# PATHOGENIC AND GENETIC VARIABILITY IN *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* AND THE MANAGEMENT OF BACTERIAL BLIGHT DISEASE

By

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**THESIS**

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**Department of Plant Pathology**

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# DECLARATION

I hereby declare that this thesis entitled **"Pathogenic and genetic variability in *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* and the management of bacterial blight disease"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other university or society.

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# CERTIFICATE

Certified that this thesis, entitled **"Pathogenic and genetic variability in *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* and the management of bacterial blight disease"** is a record of research work done independently by **Sri. S.M. Purushothaman (2006-21-110)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the advisory committee of **Mr. S.M.Purushothaman** (2006-21-110) a candidate for the degree of **Doctor of Philosophy in Agriculture,** with major field in Plant Pathology, agree that the thesis entitled **"Pathogenic and genetic variability in *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* and the management of bacterial blight disease"** may be submitted by **S.M.Purushothaman** in partial fulfilment of the requirement for the degree.

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Dedicated
to my
beloved
family

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# *Introduction*

# INTRODUCTION

Rice is grown in tropical and subtropical regions of the world and is a staple food of around 2.7 billion people worldwide (Salim *et al.*, 2003). Asia contributes about 90 per cent of the world's rice production and India ranks first in area (43 million ha) and second in rice production (104.30 million tonnes) next to China. In Kerala, rice is cultivated in an area of 2.13 lakh ha and the annual production is 5.22 lakh tonnes, the average productivity being 2452 kg/ha (Agricultural Statistics 2010-2011). Though, Kerala is not a major contributor to India's rice production. the entire population of the state centers mainly around this cereal crop. Annually, more than 40 per cent of the world rice crop is lost due to biotic stresses like insects, pests, pathogens and weeds (Hossain, 1996). Among the various diseases of rice, bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) (Ishiyama) Swings *et al.* has been an important constraint of rice production in Asia.

Bacterial blight (BB) is one of the earliest known diseases of rice and was first noticed by the farmers of Japan in 1884 (Tagami and Mizukami, 1962). Subsequently its incidence has been reported from other countries *viz.*, Korea, Indonesia, Taiwan, China, Thailand, India, Sri Lanka, Bangladesh, Vietnam, Malaysia, Pakistan, Australia, America and Africa. In India, BB was first reported in Maharashtra during 1951 (Sreenivasan *et al.*, 1959 and Bhapkar *et al.*,1960) and later observed in other states like Andhra Pradesh, Bihar, Haryana, Kerala, Orissa, Punjab and Uttar Pradesh. In Kerala, bacterial blight was first observed in 1976 in Palakkad district and thereafter Mary (1980) reported its occurrence in epiphytotic proportion almost every year in Palakkad and Kuttanad, the rice bowls of Kerala, from where the state's 60 per cent of rice is produced.

The bacterium, *Xanthomonas oryzae* pv. *oryzae* invades the host tissue through hydathode of the leaf or through mechanical injuries of the leaf blades and multiplies in the vascular system. Two types of symptoms *viz.*, leaf blight and kresek are mainly noticed under tropical conditions. Leaf blight symptoms appear at the active tillering stage of the crop as small water soaked lesions or stripes on the leaf margins, later these lesions turn yellow, enlarge progressively and extend to one or both sides of the leaf

margins. Long wavy stripes develop covering the entire leaf margins leaving a small greenish portion in the midrib of the leaves. Later these lesions become papery white and cause drying of the entire foliage. The kresek symptoms are usually observed on seedlings in the nursery or immediately after transplanting as grayish green lesions on lower leaves. Later, these lesions turn yellow and the infected leaves roll along the midrib, wither and die. Generally bacterial blight is more destructive in Asia during heavy rains of monsoon seasons and infection can sometimes lead up to 100 per cent yield losses (Mew and Majid, 1977).

Management of bacterial blight has been carried out using chemicals and development of resistant cultivars over a long period of time. However, the application of chemicals is not always effective and may also affect the environment. Breeding for bacterial blight resistance is the most economic strategy of disease management and this has only been partially successful because of the enormous diversity in the pathogen. Hence, knowledge on the virulence spectrum and genetic variability in the pathogen population in the local region is highly vital so as to develop a resistant variety based on the prevalence of the specific pathotype in a particular area. Yet another method of management is through biological control. It is an ecology conscious, cost effective alternative strategy for bacterial blight management. This can also be used in integration with other strategies so as to offer greater levels of protection and sustainable rice production.

Antagonistic bacteria are considered as an ideal biological control agents, due to obvious reasons like rapid growth, easy handling and aggressive colonization of the rhizosphere (Weller, 1988). The other factors such as siderophore production, microbial cyanide and lytic enzymes may also play a significant role. Many of the antagonistic bacteria may function as plant growth promoting rhizobacteria (PGPR), contributing to the enhancement of plant growth (Kloepper and Schroth,1978).The deployment of such bacterial strains for biological disease suppression confer additional advantages to plant systems by promoting plant growth (Glick *et al.*, 1999). Diverse mechanisms are known to be involved in enhancement of plant growth and health. This knowledge has aroused

enormous interest in biological control and favours its development as a sound, ecology conscious strategy for disease management.

With these points in view, the present study was taken up with the following objectives:

- Survey, collection and isolation of different isolates of *X.oryzae* pv.*oryzae* from disease prone areas
- Studies on the cultural, morphological and biochemical characters of the collected isolates
- Studies on pathogenic variability of *X.oryzae* pv.*oryzae* isolates
- Studies on genetic variability of different categories of isolates
- *In vitro* sensitivity of antibiotics to different isolates of the pathogen
- Isolation and enumeration of rhizo and endosphere bacteria in rice
- *In vitro* antagonistic activity of rhizo and endosphere bacteria against the pathogen
- Evaluation of selected antagonists, agro chemicals and organics under *in planta* condition
- Compatibility studies of antagonists with plant protection chemicals recommended in rice as per POP
- Study of mode of action of selected antagonists
- Field trial on the management of bacterial blight of rice

# Review of Literature

## 2. REVIEW OF LITERATURE

Rice is the most important staple food in Asia where it provides 35-80 per cent of total calorie intake (IRRI, 1997). This crop is affected by many diseases, among which bacterial blight (BB) has gained more attention due to its severity in the field and consequent yield loss.

### 2.1. Bacterial blight

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* (*Xoo*) is a common and destructive disease of rice which threats rice production in both temperate and tropical rice growing regions, due to its high epidemic potential. The disease is prevalent in irrigated and rainfed lowland rice growing areas in Asia, known to occur at any stages of the crop starting from seedling stage to the maturity of the crop (Mew, 1987). In young seedlings, the disease manifests as kresek phase, characterized by the leaves turning into grayish green, wither suddenly and roll upwards (Goto, 1992 and Nyvall,1999). Wilting of the young leaves followed by drying the whole seedlings occur within two to three weeks due to blockage of the translocation. Leaf blight symptom is generally observed from maximum tillering to flowering stages. This symptom is characterized by the development of water soaked lesions at the margins which enlarge both in length and width leading to yellowing and drying of the leaves with a characteristic wavy margin. Bacterial ooze, as yellowish spherical masses may be seen on the margins or veins of the freshly infected leaf under moist conditions (Muneer *et al.* 2007). The rapid spread of the disease in many parts of the country was favoured by the introduction of highly susceptible variety Taichung Native (TN-1) (Singh, 2001). Because of widespread cultivation of high yielding but susceptible rice cultivars and indiscriminate use of nitrogenous fertilizers, bacterial blight has become one of the serious diseases of rice in Asia.

### 2.1.1. Yield loss

Bacterial blight is one of the most serious diseases of rice causing huge yield losses in almost all the rice growing countries of Asia. Rangaswamy and Rajagopalan (1973) observed yield loss of 50 per cent in rice crops raised during monsoon season. According to Mew and Majid (1977) the yield losses were upto 100 per cent when bacterial blight infection occurred at active tillering stage. The major epidemics during the sixties and the epidemic in the Punjab state of India are well documented examples of extensive yield losses even up to the extent of 65-95 per cent due to the disease (Reddy, 1980). Adhikari and Mew (1991) recorded the yield loss up to 26 per cent in Nepal and the losses might be higher during severe epidemics. Mew *et al.*, (1993) reported that yield losses about 10 – 20 per cent were common, whereas 50 – 70 per cent was recorded in severely infected conditions. Adhikari *et al.* (1999a) reported that the total blighting of leaves or complete wilting of the affected tillers lead to unfilled grains and yield losses were mainly due to reduction in the number of tillers, number of grains per panicle and 1000-grain weight. Rajarajeswari and Muralidhran (2006) also reported yield loss ranging from 31 to 44 per cent in India.

### 2.1.2. The pathogen

Bokura (1911) isolated the bacterium from the infected rice leaves and classified the bacterium as *Bacillus oryzae*. The bacterium was renamed as *Pseudomonas oryzae* and later *Xanthomonas oryzae* (Ishiyama,1922). It was reclassified as *X.campestris* pv.*oryzae* (Dye,1978). In 1990, the pathogen was elevated to a species status and was named as *Xanthomonas oryzae* pv. *oryzae* (Swings *et al.*, 1990). *X.oryzae* pv.*oryzae* is a Gram negative rod, round ended, non spore forming and with single polar flagellum. Individual cells vary in length from 0.7 µm to 2 µm and in width from 0.4 µm to 0.7 µm. Bacterial cells produce capsular extracellular polysaccharide (EPS) and are important for the formation of droplets of bacterial exudate from infected leaves. Colonies on solid media are circular, convex, mucoid and yellow in colour due to the production of the pigment - xanthomonadin (David *et al.* 2006).

**2.2. Studies on pathogenic variability of *Xoo***

Consequent upon the failure of controlling this disease through chemicals, host resistance was given priority in disease management strategy and thus, breeding for resistance has become as integral part of the rice improvement programme. The wide variability in virulence among the bacterial strains has become a major constraint in resistance breeding programme. Sincere attempts have been made worldwide to group bacterial strains in to virulence groupings through differential varieties and pathotypes were identified in Japan (Ezuka and Horino, 1974; Noda *et al.*, 1990; Kaku,1993), in IRRI, Philippines (Mew and Vera Cruz, 1979; Mew *et al.*,1982), in Indonesia (Yamamoto *et al.*, 1977) and in India (Devadath and Padmanabhan, 1969; Gupta *et al*,1986; Nayak and Reddy, 1993).

Ogawa (1993) suggested the strategy of monitoring the race distribution of *Xoo* using near isogenic lines (NIL) and proposed a set of differentials possessing known genes for resistance. Shanti *et al.* (2001) observed nine pathotypes from the strains collected from Orissa and Madhya Pradesh. Among the population analyzed, the Orissa population was most diverse consisting five out of nine races. Singh *et al.* (2003) studied the pathogenic variability of 693 *Xoo* isolates from Punjab and 17 different reaction patterns were observed on a set of near isogenic lines in IR 24 genetic background. Resistance genes xa8 and Xa21 were the most effective followed by xa5 and Xa7 against *Xoo* isolates prevalent in northern India. Different genes in combinations (xa5+xa13, xa5+Xa21 and xa5+xa13+Xa21) provided better protection against all the isolates of the pathogen than with the component genes. Suparyono *et al.* (2004) studied the pathogenic variability of 117 isolates of *Xoo* collected from Java on five rice differentials and grouped the isolates as pathotype III, IV and VIII based on disease reactions. Shanti and Shenoy (2005) tested 11 near isogenic lines and nine gene pyramids against 10 isolates of *Xoo* and concluded that the effectiveness of genes vary in different regions and to have an effective deployment of gene combinations, regional information on the performance of individual gene is a pre requisite.

Jalaluddin *et al.* (2005) studied the pathogenic variability of 35 Bangladesh isolates of *Xoo* on 11 near isogenic lines and found the existence of 23 races. Jeung *et al.* (2006) reported that the Korean *Xoo* populations were highly different from other countries and found that the pyramid line containing genes Xa4, xa5 and Xa21 would be the most promising and valuable genotype for improving Korean japonica cultivars for bacterial blight resistance. Dinh *et al.* (2008) evaluated 41 isolates of *Xoo* from Mekon delta on 10 rice differentials containing single resistant genes and found the presence of six races. Nayak *et al.* (2008) studied the pathogenic variability of 52 strains of *Xoo* from 12 rice growing states of India on 16 rice genotypes possessing known genes for resistance and found that the pathogenic variability was high in the pathogen population from Gujarat, Punjab, Madhya Pradesh, Uttar Pradesh and Tamil Nadu. Nayak *et al.* (2009) studied the pathogenic variability of 52 *Xoo* isolates on five Indian differentials and were grouped in to six clusters and designated as pathotypes 1, 4, 7, 14, 15 and 16, following a standard computer generated virulence pattern chart. The most virulent pathotype was distributed over four eastern states of India, namely Andhra Pradesh, Orissa, West Bengal and Bihar. Jyufuku *et al.* (2009) studied the pathogenic variability of 57 strains of *Xoo* obtained from Asian countries *viz.*, India, Indonesia, Malaysia, Thai, Taiwan and Philippines and were grouped into 13 races based on their reaction on international differential lines. They also found that three strains from India and two strains from Indonesia were virulent to the resistant gene xa5. Shanti *et al.* (2010) studied the pathogenic variability of *Xoo* isolates and found that greater variation existed in the virulence patterns. Among the genes and their combinations studied, the four-gene combination (*Xa4+xa5+xa13+Xa21*) was found more resistant against the isolates. Lore *et al.* (2011) studied the pathogenic variability of *Xoo* isolates from Punjab and adjoining north western states of India and found that the pathogen was highly variable and classified the isolates into seven pathotypes (PbXo-1 to PbXo-7). Among them, PbXo-3, PbXo-4 and PbXo-7 were the most dominant and virulent pathotypes and PbXo-1, PbXo-2, PbXo-5 and PbXo-6 were least virulent ones. Chen *et al.* (2012) studied the pathogenic variability of 218 isolates obtained from different elevations ranging from 150 to 2600 m in southwest China on six near isogenic lines, each containing a single major gene and grouped them into 18 pathotypes and found that the pathotype-9, predominated in low and mid elevations and were virulent to all resistance genes, including Xa2, Xa3, xa5, xa13, Xa14 and Xa18. However, pathotype-2 was predominant

at high elevation and was virulent to Xa18 only. They observed that there were significant trends of virulence of isolates from low to high with the elevation from high to low.

### 2.3. Genetic variability of *Xoo*

Knowledge on the genetic variability of the *Xoo* population is very important for developing a resistant variety. The population structure of the pathogen needs to be monitored and understood to guide the selection and development of resistant genes. Molecular techniques have provided abundant genetic markers that can be used to assess the genetic structure of field populations of *Xoo* (Leung *et al.*, 1993). Adhikari *et al.* (1995) evaluated the population structure of 308 stations of *Xoo* obtained from China, Indonesia, India, Korea, Malaysia, Nepal and Philippines using RFLP marker with two DNA sequences from *Xoo* (IS1112 and avr Xa10). Based on the consensus of three clustering statistics, the collection formed five clusters and each cluster consists of more than one pathotype. Similar probes were used to detect the genetic diversity of the *Xoo* population in India (Yashitola *et al*; 1997). Vera Cruz *et al.* (1996) attempted to compare the efficiency of RFLP and REP-PCR for detecting the variations in the pathogen population from Philippines and observed that Box primers used in REP-PCR detected the least polymorphism and REP primers detected the most. The use of data from REP-PCR with two primer sets (ERIC and REP) and RFLP with one probe (IS1113) allowed higher level of detection than either probe/primer set or technique alone.

Rajabhosale *et al.* (1997) observed a high level of genetic polymorphism among Indian isolates of *Xoo* using hyper variable probes such as microsatellite oligonucleotide, probe (TG) 10, a human minisatellite probe, PV47, an avirulence gene probe, avr Xa10 and a repeat clone, pBS101. These DNA probes detected multiple loci in the bacterial genome generating complex DNA fingerprints and differentiated all the bacterial isolates. Cluster analysis based on hybridization patterns using all of the above probes showed five groups at 56 per cent similarity. George *et al.* (1997) conducted studies with two outwardly directed primers complimentary to sequences in IS1112, a repetitive element isolated from *Xoo* were used to fingerprint the DNA from a set of 71 bacterial blight pathogen strains using polymerase chain reaction (PCR), PCR-based restriction analysis (PBRA) and ligation-mediated PCR (LMPCR) and revealed useful polymorphisms among individual strains.

Adhikari *et al.* (1999b) reported the genotypic diversity in 171 strains of *Xoo* collected from eight rice growing zones of Nepal. Thirty one molecular haplotypes were distinguished using two polymerase assays. Gupta *et al.* (2001) analysed 16 isolates of *Xoo* representing different geographical locations in India with OPA and OPK series primers, IS1112 based primers, PJEL1 and PJEL2. The data using RAPD-PCR and IS1112 based PCR approaches revealed their potential in rapid identification of isolates for the assessment of genetic variation in the Indian pathogen population. The OPA and DPK series RARD primers grouped the isolates into two clusters, whereas RAPD-PCR (7 primers) and IS1112-PCR (2 primers) grouped them into five different clusters. Shanti *et al.* (2001) differentiated *Xoo* isolates from three regions in eastern India into 17 haplotypes by using the JEL1 and JEL2 primers. Singh *et al.* (2003) characterized 693 isolates of *Xoo* using the PCR based primers PJEL1 and PJEL2, and the pathogen populations were grouped into 97 haplotypes based on DNA banding patterns. An un-weighted pair-group method using arithmetic averages (UPGMA) indicated a high level of diversity in the pathogen isolates (51 lineages of *Xoo* at a 70% similarity level). Among these, lineages 5, 7, 27 and 29 were widely distributed and others were localized in the northern region of India.

Jalaluddin *et al.* (2005) studied PCR based DNA fingerprinting of 20 selected Bangladeshi strains and five Japanese races of *Xoo* and found that most of the Bangladeshi strains belonged to group I and a few Bangladeshi strains and five Japanese races belonged to group II. Nayak *et al.* (2008) grouped 52 strains of *Xoo* collected from 12 rice growing states of India, into 13 clusters based on genetic distance. Lore *et al.* (2011) studied the genetic variability of *Xoo* using RAPD-PCR and IS1112 based PCR produced unique DNA fragments specific for different pathotypes that lead to the rapid assessment of genetic variation in the pathogen population. Yong *et al.* (2011) studied the genetic variability of 103 China isolates of *Xoo* using the ERIC and BOX primers. Dendrograms were generated from the combined data of the above primers using the UPGMA analysis and found that extensive genetic variability existed within the pathogen population.

Chen *et al.* (2012) studied the genetic variability of 218 isolates obtained from different elevations ranging from 150 m to 2600 m in southwest China using ERIC and J3 primers and grouped them into 56 molecular haplotypes. Shahrestani *et al.* (2012) studied the genetic

variability of 60 *Xoo* isolates from Iron using the RAPD markers and found the presence of greater genetic variation in the pathogen population and grouped them into three clusters at a similarity index of 0.60. Lakshmi and Rabindran (2012) studied the genetic variability of 17 isolates of *Xoo* obtained from different parts of Tamil Nadu using RAPD-PCR and IS 1112 based PCR analysis and grouped them into two clusters, A and B. The percentage similarity coefficient values ranged from 0.18 to 0.74 and all the *Xoo* isolates showed variability at molecular level.

## 2.4. Compatibility studies of biocontrol agents with agrochemicals

In present day's agriculture, environmental-friendly sustainable plant protection measures like the use of organics and bioagents have gained momentum. Still, agrochemicals cannot be avoided. Hence, integrated management by combined use of bio agents and chemicals have attracted much attention as a way to obtain synergistic or additive effects in managing plant diseases.

The idea of combining biocontrol agents (BCA) and fungicides are widely exploited for the development and establishment of desired microbes in the rhizosphere (Papavizas and Lewis, 1981). Lindaw *et al.* (1996) reported that combination of *P. fluorescens* with antibiotics in the control of fire blight and frost injury to pear. Vidhyasekaran and Muthamilan (1996) reported the compatibility of carbendazim and Thiram with *P. fluorescens*. Combined effect of pesticides and bacterial biocontrol agents other than *Pseudomonads* were reported by Heydari *et al.* (1997) and Chenzhiyi *et al.* (1998). Elkins and Lindow (1999) reported that mancozeb had no detrimental effect on *P. fluorescens* A506 when applied at least five days before or after the application of bioagent. Naar and Kecskes (1999) opined that antagonism of BCA was highly influenced by the addition of fungicides.

Compatibility of systemic fungicides *viz.*, thiophanate methyl and carbendazim with *P.fluorescens* was tested against *Colletotrichum falcatum* and found that growth of *P.fluorescens* (11 strains) was not affected up to 500 ppm of both the fungicides under *in vitro* and *in vivo* tests (Malathi *et al.*, 2002). Mathew (2003) also reported the compatibility of *P.fluorescens* with mancozeb and carbentazim. Nallathambi and Thakore (2003) observed that combined treatment of *P. fluorescens* and any one of the following fungicides *viz.*, thiophonate methyl, Captan or

Aldicin at 50 ppm resulted in 60 per cent more disease suppression than tested individually. Bhavani (2004) observed the compatability of *P. fluorescens* with Akomin-40, Indofil-M-45 and Bavistin whereas among the copper fungicides, Bordeaux mixture was more inhibitory to the antagonists followed by Kocide and Fytolan. *In planta* studies with *Pseudomonas* strains confirmed the compatibility with the fungicides tested *viz.*, metalaxyl, mancozeb, potassium phosphonate and carbendazim. However, the bacterial strains were not compatible with copper oxychloride (Paul, 2004). Kumar *et al.* (2011) studied the compatibility of *Bacillus subtilis* MB 600 (commercial formulation namely Integral) with eight fungicides and found that the rice sheath blight bacterial antagonist had good compatibility with carbendazim and azoxystrobin up to 400 ppm.

Five rhizobacterial isolates of fluorescent *Pseudomonas* spp. were tested for their sensitivity to various antibiotics by Samanta and Dutta (2004). They found that the strain MPf-1 was insensitive to all the tested antibiotics viz., Pencillin G (10 units), streptomycin (10 µg), gentamicin (10 µg), norfloxacin (10 µg), kanamycin (30 µg) and nalidixic acid (30 µg), whereas the strain MPf-2 was highly sensitive to only norfloxacin and the isolate P-2 was highly sensitive to norflaxicin, gentamycin and streptomycin.

Mathew (2003) reported the compatibility of *P. fluorescens* with the insecticides *viz.*, imidacloprid, etofenprox, chlorpyriphos and triazophos at the recommended doses. Thankamani *et al.* (2003) noticed that application of *P. fluorescens* + VAM in black pepper along with Phorate and copper oxychloride resulted in significantly higher number of leaves, maximum length of roots, leaf area and total biomass which indicated the compatibility of phorate with *P. fluorescens*. Among the insecticides, two lower concentrations of Sevin, Ekalux, Nuvacron and Endosulfan were compatible with the *Pseudomonas* strains compared to their higher concentrations. Phorate at all concentrations was found compatible with the selected isolates (Bhavani, 2004). According to Paul (2004) the selected *Pseudomonas* strains were found compatible with chlorpyriphos, quinalphos, dimethoate and Phorate.

Bhavani (2004) observed that the fertilizers rajphos and muriate of potash were compatible with the antagonists, whereas urea, ammonium chloride and ammonium sulphate showed varying levels of inhibition, indicating their partial compatibility.

## 2.5. Management of bacterial blight of rice

### 2.5.1. Biological control

Rhizosphere bacteria present in large numbers on the root surfaces and in root interiors as endophytes have proven their ability as efficient biocontrol agents. Consequently, intimate association between bacteria and host plants are formed without harming the plant. These beneficial free-living soil bacteria termed as plant growth promoting rhizobacteria (PGPR) have the capablility to reduce the plant diseases (Kloepper *et al.*, 1980a). According to Kloepper *et al.* (1992), rhizobacterial strains suppress the pathogens, promote the crop growth, increased the availability of nutrients and also induced systemic resistance.

Nayar and Vidhyasekaran (1998) studied the management of rice bacterial blight disease using the p1 strain of *P. fluorescens* and found that the combined application of seed treatment, seedling root dipping and foliar spray controlled the bacterial blight by 60-70 per cent under field conditions. When *P. fluorescens* (Pf1) was treated against bacterial blight disease, a sharp increase in lignification and activities of peroxidase, phenylalanine ammonia-lyase and 4-coumarate-CoA ligase were observed (Vidhyasekaran *et al.*, 2001). Manav and Thind (2002) reported that the two rhizobacteria *viz.*, *B. subtilis* and *P. fluorescens* could inhibit the bacterial blight pathogen *Xoo* under *in vitro*. The evaluation of these antagonists in pot experiments as seed treatment, seedling dip and foliar sprays significantly reduced the disease intensity. Jigang *et al.* (2005) reported a novel rhizoplane Diazotrophic Plant Growth Promoting Bacterium (PGPB) *Delftia tsuruhatensis* strain HR4 from North China inhibited rice bacterial blight pathogen *Xoo* by 78 per cent under *in vitro* and found that it could reduce the disease 30 per cent by seed soaking and 24 per cent by foliar spraying in the cultivar Nonghu 6 under green house condition. Velusamy and Gnanamanickam (2003) screened 637 fluorescent bacterial strains obtained from rice rhizosphere samples collected from various places of Karnataka, Kerala and Tamil Nadu against *Xoo*. They found 278 strains showed antibiosis in laboratory bioassays and 27 strains could produce 2,4-diacetyl phloroglucinol (2,4-DAPG) and a strain from Pattambi (PTB-9) on seed treatment, seedling dip and two foliar sprays could reduce the bacterial blight severity by 64.50 per cent under field conditions.

Jayalakshmi *et al.* (2010) studied the effect of different methods of application of *P.fluorescens* against bacterial blight disease incidence, growth promotion and yield

improvement in direct seeded wet sown rice, under field condition and found that application of *P. fluorescens* as seed treatment was found to be highly effective by recording minimum bacterial blight incidence (1.11%) and maximum yield (4.1t/ha) where as the control plots recorded the maximum disease incidence (60%) with minimum yield (1.2 t/ha). Mondal *et al.* (2010) studied the efficacy of *P. fluorescens* strain MBPF-01 alone or in combination with nanocopper against *Xoo* under *in vitro* and *in vivo* and observed that one spray of nanocopper followed by spraying of *P. fluorescens* strain MBPF-1 at seven days interval could reduce the disease by 70 per cent over control by inducing resistance against *Xoo*. Gangwar and Sinha (2010) studied the antagonistic activity of 19 isolates of fluorescent pseudomonads against the *Xoo* under *in vitro* and found that maximum inhibition zone (18.80 mm) was shown by the isolate FLP 88 followed by FLP 85 (17.30 mm), FLP38 (17 mm), Pf 83 (15.50 mm) and FLP3 (15.30 mm). Pupakdeepan and Prathuangwong (2010) developed a powder formulation using a rhizosphere bacterial strain X46 against bacterial blight disease in rice and revealed that seed treatment followed by two foliar sprays resulted in 71.60 per cent decrease in bacterial blight severity under greenhouse condition.

Chithrashree *et al.* (2011) evaluated seven PGPR strains of *Bacillus* for growth promotion and induced systemic resistance in rice against *Xoo* and found that among the seven strains tested as fresh suspensions, talc and sodium alginate formulations under laboratory and greenhouse conditions. Maximum germination of 86 per cent was recorded after seed treatments with fresh suspension of *B. subtilis* GBO3 followed by 85 per cent germination treated with *B. pumilus* SE34 in comparison to 71 per cent germination in untreated controls. Similarly, the maximum vigour index of 1374 was obtained by seed treatment with fresh suspensions of *B. subtilis* strain GBO3 followed by treatments with strain SE34 with vigour index of 1323 in contrast to an index of 834 in untreated control. Among the treatments, seed treatments with fresh suspension of seven strains of *Bacillus* spp. resulted in better germination and vigour than talc based or sodium alginate formulations. They also found that seed treatment with fresh suspension of strain SE34 gave 71 per cent protection, followed by *B. subtilis* GBO3 and *B. pumilus* T4 with 58 per cent and 52 per cent protection respectively.

Gangwar and Sinha (2012) studied seven isolates of fluorescent pseudomonads *viz.*, FLP 84, FLP 88, FLP 90, FLP 85, FLP 2, FLP 3 and FLP 28 and *P. fluorescens* isolate 83 (Pf 83)

against *Xoo* under greenhouse condition and found that all the isolates of fluorescent pseudomonads were found significantly effective in reducing disease severity compared to control. The maximum reduction was shown by Pf83 and FLP 85 (62.18%), followed by FLP 90 (60.77%) and FLP 88 (58.65%). FLP 28 was the least effective, showing 49.46 per cent reduction in disease severity. Isolate FLP 88 was the best in increasing grain yield (60.74%), followed by FLP 84 (52.35%) and Pf 83 (50.67%). The isolate FLP 88 also recorded the highest 1000 grain weight of 26.97 per cent, followed by Pf83 (26.30%) and FLP 84 (25.30%).

### 2.5.2. Organic products

Mary *et al.* (1986) observed that foliar spray of cow dung extract (20 g /l) was highly effective against bacterial blight and was equivalent to Penicillin (100 ppm), paushamycin (250 ppm) and streptomycin (100 ppm). Sreekumar and Nair (1990) reported that, cow dung extract was better than the chemical treatments *viz.*, terramycin, streptocycline and bactrinol-100 under pot culture conditions. Das *et al.* (1998) reported the efficacy of natural products *viz.*, fresh cow dung and hing (gum of asafoetida) along with an antibiotic plantomycin to manage the bacterial blight of rice under field conditions over a period of three seasons. This study conducted in Bhubaneswar and found that foliar spray of cow dung suspension at 50 kg /ha reduced the incidence of bacterial blight significantly showing the lowest percentage of leaf area infection (18.53%) compared to unsprayed control (38.03% ) with increased mean grain yield of 4136 kg/ha.

Sinha and Sinha (2000) studied the efficacy of fresh cow dung, neem cake (150 kg/ha), neem cake suspension (5%), Neem Gold (20 ml/l) along with two chemical treatments *viz.*, Blitox 50 (0.25%) + streptocycline (50g/ha) and streptocycline (250g/ha). They found that Neem Gold was effective in reducing the disease severity next to the chemical treatment Blitox 50 (0.25%) + streptocycline (50g/ha). Sible *et al.* (2004) found that 20 per cent cow dung water extract (CDWE) spray one day before inoculation of the *Xoo* could reduce bacterial blight disease by 50 per cent. They also observed that the pre treatment of rice leaves with CDWE as foliar spray induced the defense enzymes *viz.*, peroxidase,'chitinase and beta-1, 3-glucanase in rice plants.

Jabeen *et al.* (2009) studied the antibacterial activity of 63 crude plant extracts against *X.oryzae* pv.*oryzae* and found only 10 aqueous extracts *viz.*, *Thuja orientalis*, *Prunus domestica*, *Citrus limon*, *Allium sativum*, *Vitis vinefera*, *Mangifera indica*, *Phyllanthus emblica* and *Terminalia chebula* showed maximum inhibitory activity. On detached leaves in glasshouse and field assay, two potential plant extracts *viz.*, *Allium sativum* and *Citrus limon* showed maximum inhibitory activity against bacterial blight in rice. *Curcuma longa* and *Allium cepa* leaf extracts ranked next in reducing the disease incidence. Murugan *et al.* (2012) studied the antibacterial activity of cow urine and extracts of *Pongamia pinnata* seeds (aqueous and solvent fractions) against *X.oryzae* pv.*oryzae* in comparison with streptomycin sulphate (30 µg) and revealed that all fractions of *Pongamia pinnata* seeds and cow urine were effective and showed 10 to 13 mm zone of inhibition and phytochemical analysis revealed the increased presence of terpenoids, quinines, tannins and phenols.

**2.5.3. Chemical control**

Swarup *et al.* (1965) observed that mancozeb (2000 ppm) and $HgCl_2$ (1000 ppm) could inhibit *Xoo* at the maximum level. Desai *et al.* (1967) reported that 17 species of *Xanthomonas* were inhibited by streptocycline at concentration of 25 to 250 ppm under *in vitro*. Shetty and Rangaswamy (1968) found that streptocycline at 25- 50 ppm was inhibitory to three isolates of *Xoo* and was lethal at 50-100 ppm. Balaraman and Rajagopalan (1978) found that tetracycline gave the maximum inhibition zone followed by ledermycin, erythromycin and chloramphenicol. Chauhan and Vaishnav (1980) observed that the best method for control of *Xoo* was due to the application of streptocycline along with copper containing compounds. Durgapal (1983) found that the most suitable means for controlling *Xoo* by submerging the rice seedlings for 24 h in streptocycline before transplanting. Sivaswamy and Mahadevan (1986) studied the effect of bleaching powder on bacterial blight of paddy and found that application of bleaching powder at the concentration > 100 µg/ml could reduce the survival and population of *Xoo*.

Sreekumar and Nair (1990) studied the effect of spraying with Bactrinol-100, tetracycline, streptocycline and cow dung extract for the control of bacterial blight disease under field condition. The reduction in disease intensity was maximum after spraying with terramycin followed by Batrinol-100, streptocycline and cow dung extract. However, the increase in yield

obtained by spraying with Bactriniol-100 and cow dung extract was higher than that of plants sprayed with streptocycline. Further, the increase in thousand grain weight and straw yield was maximum in plants sprayed with cow dung extract. Thind and Mehra (1992) studied the efficacy of bleaching powder (250µg/ml), Plantomycin (500µg/ml), streptocycline (500µg/ml) and zinc sulphate (2%) as pre-planting nursery dip treatments in glasshouse and field trials and revealed that all the treatments except zinc sulphate could reduce the bacterial blight disease intensity.

Mehra *et al.* (1994) studied the seed treatment efficacy of bleaching powder (100µg/ml), Blitox-50WP (62.50 mg dissolved in 15 ml of acetone for 25 g of seed), sterptocycline (100µg/ml) and zinc sulphate (2%) for the management of bacterial blight of rice *Xoo* and found that the treatments could reduce the intensity of disease but did not increase yield. Mukhopadhyay (1995) stated that biological seed treatment, integrated with suitable fungicide was found highly effective and resulted in enhanced growth performance compared to biological control or chemical control alone. Mary *et al.* (2001) reported the prophylactic and curative efficacy of streptocycline (500 ppm), streptomycin + oxytetracycline (1:9, 250 and 500 ppm), Bactrinol-100 (500 ppm) and cow dung extract (20 g/l) against the bacterial blight of rice and revealed that reduction in bacterial blight incidence and an increase in straw and grain yields were observed in all treatments except Bacterinol-100. The percentage of disease index was lower for curative (40.16) than for prophylactic (42.22) spraying. Manav *et al.* (2001) studied the *in vitro* efficacy of five chemicals against *Xoo* by paper disc diffusion method and found that copper sulphate and monocrotophos could inhibit the growth of the bacterium.

Yasin *et al.* (2007) tested the efficacy of antibiotics *viz.*, Penicillin @ 1g/l, streptomycin sulphate @ 1g/l individually and in combinations, copper oxychloride @ 3g/l alone and in combination with antibiotics and Bordeaux mixture (4:4:50) alone against bacterial blight of rice. Although all applications individual or in combination, induced reduction in bacterial blight disease percentage in two years. Significant reduction was recorded in treatment sprayed with Bordeaux mixture (4:4:50) alone. The previously recommended chemical copper oxychloride, was matched with Bordeaux mixture in disease control and increased yield. Biswas *et al.* (2009) conducted experiments in Kanpur, U.P, India during 2003, 2004 and 2005 to evaluate the efficacy of the following treatments against bacterial blight of rice. Seed treatment (ST) with streptocycline and foliar spray (FS) of streptocycline + blue copper (100+500 or 50+500 ppm),

ST with streptocycline+ FS of paushamycin + blue copper (100+500 or 50+500 ppm), ST with streptocycline + FS with streptocycline + FS with paushamycin (100ppm), ST with streptocycline + FS of kitazin (1000ppm), ST with streptocycline, FS with streptocycline (100 ppm) and FS with paushamycin. All the treatments significantly reduced the disease severity. ST with streptocycline (100 ppm) and three foliar applications of streptocycline in combination with copper oxychloride (100+500 ppm) was the most effective in minimizing the disease incidence with highest yield. Patel *et al.* (2009) conducted field experiments to evaluate different chemicals, antagonists and botanicals against bacterial blight of rice in South Gujarat and found that streptomycin sulphate + copper oxychloride was found superior in reducing bacterial blight intensity, increased grain, straw yields and 1000 grain weight. The next effective treatment was copper oxychloride + *P. fluorescens*.

Chaudhary *et al.* (2012) studied the effectiveness of some fungicides and antibiotics for the control of bacterial blight in rice at Adaptive Research Farm, Gujranwala, Pakistan and farmers' fields (Wazinrabad, Gujranwala) during 2006-2008. The Bordeaux mixture (2.5:2.5:300 *ie.*, 2.5kg $CuSo_4$, 2.5 kg quick lime and 300 l water/ha), copper hydroxide 50% (1.25kg/ha), oxytetracycline (38.67 and 42.67%) and streptomycin (36.89 and 39.55%) were applied as compared to control (56 and 63.45%). The treatments also gave maximum yield *i.e.*, 4.24, 4.27 and 4.34 t/ha at research farm and 3.45, 3.46 and 3.52 t/ha at farmer's field respectively. Bordeaux mixture alone gave maximum net return (Rs.9375/ha) followed by its combination with oxy tetracycline (Rs.8975/ha) and streptomycin (Rs.8375/ha). Thimmegowda *et al.* (2012) studied the efficacy of antibiotics and biorational pesticides against bacterial blight of paddy under *in vitro* condition. The result revealed that streptocycline 1000 ppm gave maximum inhibition zone (25.00 mm) followed by streptocycline + copper sulphate 1000 ppm (24.33 mm). Under field studies, it was found that streptocycline + copper oxychloride was found best with least per cent disease index. Highest grain yield was recorded in streptocycline + copper oxychloride treatment @ 0.025+0.1%, followed by Bactrinashak @ 0.04 and 0.03%.

## 2.7. Mechanisms of action of biocontrol agents

### 2.7.1. Growth promotion and disease suppression

The efficacy of bacterial antagonists such as *P. aeruginosa* and *P. fluorescens* in promoting growth and suppressing *R. solanacearum* of tomato bacterial wilt has been reported by various workers (Manimala, 2003 and JianHua *et al.*, 2004). Paul (2004) reported that seven rhizobacterial strains significantly enhanced the growth in treated black pepper in terms of shoot and root biomass and also in terms of height of the plant and among them, five strains significantly reduced the root rot upon challenge inoculation. Rajendran *et al.* (2006) reported that cotton plants inoculated with endophytic bacteria in the greenhouse had a significantly higher yield of bolls and kapas than untreated plants. Tripathi *et al.* (2006) isolated five endophytic bacteria from rice plants which were found as siderophore producers and among them three were able to produce indole acetic acid. Nair *et al.* (2007) studied four rhizobacterial strains with acibenzolar-*S*-methyl (ASM) a chemical activator, which suppressed foliar blight of amaranthus caused by *R. solani.* The study revealed that reduction in shoot length due to application of ASM was reduced significantly when plants were treated with rhizobacterial strain *P.fluorescens* PN026R. Combined use of plant growth promoting rhizobacteria (PGPR) and ASM was found to be beneficial as the growth retardation effect of the plant defence activator was reduced by the growth promoting ability of the rhizobacteria.

Alive *et al.*(2008) studied 120 PGPR isolates against potato wilt causing virulent strain of *Ralstonia solanacearum* (PPRC-Rs) under *in vitro* and found that the strains *viz.*, PFMRI, BS-DFS, and PF9, significantly reduced wilt incidence by 82.7, 66.2, and 65.7per cent, respectively compared to the control. During the sole application, the strains significantly increased plant height by 35.6, 45.9, and 45 per cent, and dry matter by 111, 130.40 and 129 per cent, respectively compared to non-bacterized control. In the presence of the pathogen strain, PFMRI, BS-DFS, and PF9 increased plant height by 66, 50 and 48.20 per cent and dry matter by 153.80, 96.80, and 92.50 per cent, respectively compared to the pathogen treated control. Ardakani *et al.* (2010) isolated two strains of PGPR *viz.*, *P. fluorescens* Q18 (B1) and CKK-3 (B2) from rhizosphere soil of cotton and found that they could increase the seedling height, root length, seedling dry weight and root dry weight more effectively than the control. Minaxi and Saxena (2010) isolated PGPR strains *viz.*, *Pseudomonas fluorescens* BAM-4 and *Burkholderia cepacia* BAM-6 that could inhibit the charcoal rot of moong bean caused by *Macrophomina phaseolina.*

Seed bacterization showed a significant increase in seed germination, shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, leaf area and rhizosphere colonization. Yield parameters such as pods, number of seeds, and grain yield per plant were also enhanced significantly compared with control. Kumar *et al.* (2011) found that the PGPR strains *viz.*, *Bacillus amyloliquefaciens* AP 219, *B. subtilis* MBI 600 and *B. subtilis* AP 52 could suppress the sclerotial germination of rice sheath blight pathogen *Rhizoctonia solani* and could increase the seedling vigour. Zaccardelli *et al.* (2011) found that a PGPR strain C13 of *Pseudomonas putida* was able to improve yield and protect tomato against TSWV in field. Sadi and Masoud (2012) studied the antagonistic activity of *P. fluorescens* strain UTPF61 against the sunflower damping off caused by *Sclerotinia sclerotiorum* and found that the bacterium could control the disease as well as increased the growth and yield of sunflower under greenhouse condition. Niranjana and Hariprasad (2012) isolated 36 *Serratia* spp. form the rhizozphere soil of tomato and found that eighteen could promote the plant growth and the isolate Pan-9/c could reduce the *Fusarium* wilt incidence significantly and the application of bacterial formulation as a seed treatment increased the plant growth and yield.

### 2.7.2. Growth regulators

Direct influence of growth promotion by PGPR is attributed to the production of phytohormones *viz.*, cytokinins, IAA, gibberellins and regulatory molecules like ACC deaminase and fixation of atmospheric nitrogen (Patten and Glick, 1996). Many rhizobacteria, especially fluorescent pseudomonads are known to produce several hormones like auxins and cytokinins. This leads to certain morphological changes in plants like increased root growth, leaf expansion, shoot growth, etc.

Rubio *et al.* (2000) noticed that *Pseudomonas putida*, *P. aeruginosa*, *P. fluorescens* and *P. cichorii* produced extracellular IAA at varying concentrations, of which, *P. putida* produced 28.7 to 14.8 mg $l^{-1}$ and *P. aeruginosa* 21.2 mg $l^{-1}$. Dey *et al.* (2004) observed that fluorescent pseudomonads were the best in production of IAA and siderophore which resulted in higher pod yield, nodule dry weight, root length and pod number of peanuts than control. Paul (2004) reported that the selected strains of *Pseudomonas* spp. produce IAA and GA as was detected in chromatographic studies. Bhatia *et al.* (2005) reported that production of IAA was confirmed by

development of pink colour upon addition of phosphoric acid to culture supernatant of *Pseudomonas* sp.

Khakipour *et al.* (2008) evaluated 50 strains of fluorescent pseudomonads isolated from Iran soils for secretion of auxin compounds and found that 72% of the strains exuded at least one type of indolic auxin composites in HPLC. The amount of exuded IAA by *P. fluorescens* strains was varied from zero to 31.6 mg/litre while it was from zero to 24.08 mg/litre in *P. putida* strains. Ashrafuzzaman *et al.* (2009) isolated 10 PGPR isolates *viz.*, PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2, from the rice rhizosphere soils of Bangladesh and studied for their enhancement of rice growth and observed that the isolates *viz.*, PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induced the production of indole acetic acid (IAA), whereas only PGT3 isolate was able to solubilize phosphorus. Most of isolates could significantly increase plant height, root length and dry matter production.

Lavakush *et al.* (2012) studied the eight characterized PGPR isolates from rice *viz.*, *Pseudomonas aeruginosa* strain BHUJY12 (HQ-236532), *Pseudomonas putida* strain BHUJY14 (HQ-236533), *P.aeruginosa* strain BHUJY16 (HQ-236535), *Pseudomonas* sp. strain BHUJY19 (HQ-236519), *P.aeruginosa* strain BHUJY22 (HQ-236541), *P.putida* strain BHUJY23 (HQ-236542), *P. aeruginosa* strain BHUJY24 (HQ-236543) and *P. aeruginosa* strain BHUJY25 (HQ-236544) for IAA production and found that the bacterial strains could produce IAA ranging from 12.19 to 22.91 $\mu g\ mL^{-1}$ at 6 days incubation. Among all the strains, BHUJY23 was found maximum IAA production followed by strain BHUJY16.The PGPR strains were also showed the growth inhibition of *Rhizoctonia solani* in rice. Yuttavanichakul *et al.* (2012) studied the inhibition of *Aspergillus niger* which causes root rot disease in peanut by using 765 bradyrhizobial and 350 soil isolated plant growth promoting rhizobacteria (PGPR) strains and found that only 11 PGPR isolates were found to inhibit *A. niger* growth. All the 11 PGPR isolates could produce an auxin (indole-3-acetic acid) hormone and biofilms. IAA produced from PGPR isolates could promote peanut root growth also. Mishra and Kumar (2012) studied the IAA production of two PGPR strains from rice *viz.*, *Bacillus amyloliquefaciens* and *Bacillus subtilis* strains for their IAA production observed that *B. subtilis* was more efficient than *B.amyloliquefaciens* its capability to produce IAA and siderophore. Significant increase in rice plant growth was observed, when the strains were used as bio inoculum.

### 2.7.3. Hydrogen cyanide (HCN) / Volatile compounds

Defago *et al.* (1990) reported that suppression of black root rot of tobacco (*Thielaviopsis basicola*) and take all of wheat (*Gaeumannomyces graminis* f.sp. *tritici*) by *P. fluorescens* strain 'CHA0' was attributed to the production of HCN which was accounted for about 60 per cent of its biocontrol activity. They suggested that CHA0 was found to colonize the root cortex which might produce a stress effect in the plant leading to cyanide respiration and possible modification of tobacco metabolism resulted in enhanced host resistance. While studying the antagonistic efficacy of different strains of *Pseudomonas*, Mondal *et al.* (2000) found that *P. fluorescens* strain CRb-17 was the most effective producer of HCN and siderophore which in turn resulted the disease suppression of *Xanthomonas axonopodis malvaceraum* in cotton. Gupta *et al.* (2002) found that fluorescent *Pseudomonas* strain GRC2 produced HCN, IAA as well as siderophore in iron deficient medium.

Nagarajkumar *et al.* (2004) observed a significant relationship between antagonistic potential of *P. fluorescens* MDU2 against *R. solani* causing sheath blight in rice, as the strain was able to produce HCN, siderophore, salicylic acid besides the production of chitinase and ß-1, 3- glucanase. Paul (2004) reported that different strains of *Pseudomonas* produced different intensities of colour indicating different amounts of HCN produced and the highest was by strain IISR-6. Ahmadzadeh *et al.* (2004) and Bhatia *et al.* (2005) observed that only few strains of fluorescent *Pseudomonas* were able to produce HCN which was confirmed by the change in colour from yellow to reddish brown of the filter paper.

Datta *et al.* (2010) isolated PGPR bacterial isolates from chilli rhizosphere soil and tested for their plant growth promoting activities *viz.*, ammonia production, phosphate solubilization, siderophore production, IAA production, hydrocyanic acid (HCN) production and their antagonistic effect against plant pathogens *viz.*, *Xanthomonas* and *Fusarium* and revealed that out of 36 isolates, 32 isolates showed multifunctional PGPR activity and suggested for its exploitation in crop improvement and managing plant pathogens. Rana *et al.* (2011) screened 100 bacterial isolates from wheat rhizosphere for seed germination assay and found that ten bacterial isolates (AW1-AW10) were effective in enhancing the seed germination and further tested *in vitro* for specific PGPR traits *viz.*, IAA, siderophore, ammonia, HCN, P solubilization,

ACC deaminase activity, acetylene reduction assay and antifungal activity and found that AW5 was the promising isolate for all PGP attributes and was identified as *Providencia* sp.

Karimi *et al.* (2012) studied the antagonistic activity of six isolates of *Pseudomonas* and six isolates of *Bacillus* from chickpea rhizosphere against *Fusarium oxysporum* f. sp. *cicerii* and found that all were inhibiting the pathogen in both *in vitro* and *in vivo* experiments. Based on phenotypic properties, selected isolates were identified as *Bacillus subtilis* (B1, B6, B28, B40, B99 and B108), *Pseudomonas putida* (P9 and P10) and *P. aeuroginosa* (P11, P12, P66 and P112) and the antagonists were subjected to their ability for the production of cyanide hydrogen, siderophore, protease and indole acetic acid (IAA). The study revealed that the level of production was varied among the antagonists. Niranjana and Hariprasad (2012) isolated 36 rhizobacteria belonging to genus *Serratia* from 57 rhizospheric soil samples of different tomato growing regions of Karnataka and found that 29 isolates were found to colonize the roots of tomato and were analyzed for their plant growth promoting ability and observed that 18 isolates were found promoting the plant growth and were also able to produce IAA, ACC deaminase, chitinase, β-1,3-glucanase, solubilize phosphate, HCN and were resistant to multiple antibiotics.

Kumar *et al.*(2012) isolated 30 bacterial isolates from six French bean rhizospheric soil samples from different locations of Shimla and Solan in Himachal Pradesh and subjected them for different plant growth promotion activities *i.e.*, phosphate solublization, IAA production, ammonia production, ACC deaminase activity, HCN production and catalase under i*n vitro* and observed that twelve bacterial isolates were positive for phosphate solublization, three isolates were positive for ammonia production and two isolates were positive for HCN production and all the isolates were found to be positive for catalase activity. Five isolates were showing maximum plant growth promotion activities and were identified as *Acinetobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Micrococcus* sp., and *Pseudomonas* sp.

### 2.7.4. Siderophores

According to Schippers *et al.* (1987) siderophores are secondary metabolites produced virtually by all bacteria and fungi under iron limiting conditions, selectively chelates iron and make it unavailable to other deleterious micro organisms and soil borne pathogens, thus reducing their population. Bakker *et al.* (2002) reported that competition for iron and induced

systemic resistance (ISR) are the effective mechanisms of siderophore mediated disease suppression by *P. fluorescens*. They reported that *P. fluorescens* WCS374 produced the siderophore pseudobactin and salicylic acid (SA) at low iron availability and both these compounds are involved in ISR in radish.

Blanco *et al.* (2004) reported that all the isolates of *P. fluorescens* and *P. putida* could produce green fluorescent siderophore pseudobactin *in vitro*. According to Reddy *et al.* (2004) siderophore production was observed on the reverse side of Petri plates as green dots and also the change of colour of the medium to fluorescent green by growing in King's B medium. Storey (2005) detected fluorescent pseudomonads produced siderophores by formation of yellow halo zones in CAS plates. According to Bhatia *et al.* (2005), all the strains of fluorescent *Pseudomonas* were able to chelate $Fe^{3+}$ from chromeazurol S agar medium. Rajkumar *et al.* (2005) reported the ability of two PGPR strains A3 and S32 for the growth promotion of *Brassica juncea* under chromium stress condition and found that the microbial production of siderophores and indole 3 acetic acid (IAA) were the base for the growth promotion of *Brassica juncea* under chromium stress condition. Lavaca *et al.* (2008) reported that 37 endophytic strains of *Methylobacterium* spp. isolated from citrus were able to produce hydroxamate type of siderophore.

Naureen *et al.* (2009) screened bacterial strains from rice rhizosphere for their antagonistic activity against *Rhizoctonia solani*, the rice sheath blight pathogen and found that the antagonism was strongly correlated with the quantity of siderophores production. The study also revealed that the high siderophore producing strains *viz.*, *Bacillus subtilis* SPS2, *Bacillus cereus* Z2-7, *Enterobacter* sp. SPR7 and *Aeromonas hydrophilla* BPS10 were found to promote the growth of rice plants by the solubilisation of soil phosphates, nitrogen fixation and the production of phytohormones. These same plant growth promoting rhizobacteria (PGPR) also conferred high resistance against sheath blight, which resulted in significant yield increase in infected plants. Maleki *et al.* (2010) found that the cucumber PGPR strain *P.fluorescens* (CV6) was shown to have broad spectrum antagonistic activity against *Phytophthora drechsleri* the causal agent of cucumber root rot and found that the strain offered antibiotic activity against 11 additional plant pathogens also and could produce varying levels of siderophore and indole-3-acetic acid (IAA).

Soltani *et al.* (2012) tested 25 isolates of fluorescent pseudomonads isolated from wheat rhizosphere for their PGPR traits *viz.*, production of chitinase, salicylic acid, siderophore and hydrogen cyanide and antifungal activity against *R. solani* and found all could produce siderophore on Chrome Azurol S (CAS) agar plates with halo diameter of 0.34 -1.21. None of these isolates could produce chitinase. Salicylic acid (SA) production by the isolates ranged from 0-10.91 $g.ml^{-1}$ and a great variation in hydrogen cyanide production was also noticed and three isolates *viz.*, PA24, PA1 and PA18 showed antifungal activity against *R.solani*.

### 2.7.5. Antibiotics

Antibiotics play an active role in the biocontrol of plant diseases. According to Thomashow *et al.* (1997) antibiotics are organic low molecular weight compounds produced by microbes which at low concentrations are deleterious to the growth or metabolic activities of other microorganisms. Bonsall *et al.* (1997) reported that the antibiotic, 2,4-diacetylphloroglucinol (2,4-DAPG) produced by *P. fluorescens* Q2-87 obtained from wheat was effective against the take-all pathogen of wheat. Paul (2004) observed that two strains *P.fluorescens viz.*, IISR-6 and IISR-8 could produce pyoluteorin, indicating their role in disease suppression of *Phytophthora* disease of black pepper. Velusamy and Gnanamanickam (2003) screened 637 florescent bacterial strains obtained from rice rhizosphere samples collected from various places of Karnataka, Kerala and Tamil Nadu against *Xoo* and found 278 strains showed antibiosis in laboratory bioassays and 27 strains could produce 2,4-DAPG. Samanta and Datta (2004) reported that the antibiosis property might be the most important parameter for judging the plant growth promoting potential of indigenous rhizobacteria.

Vleesschauwer and Hofte (2007) found that the root treatment with *P. fluorescens* strain WCS374 effectively protected rice plants against both *Magnaporthe grisea* and *R. solani* due to the triggered action of siderophores, pseudobactin and salicylic acid in rice plants. Gangwar and Sinha (2010) studied the antagonistic activity of 19 isolates of fluorescent pseudomonads against the *Xoo* under *in vitro* and found that maximum percent inhibition (62.7%) of *Xoo* was observed with the 48 h old culture of Pf 83.

### 2.7.6. Efficacy of rhizobacteria in mobilizing nutrients from the rhizosphere

A large proportion of phosphorous in soil is present as insoluble form and therefore not available to the plant. The ability to convert insoluble 'P' to an accessible form like orthophosphate is an important trait for PGPR for increasing plant yields.

Dey *et al.* (2004) noticed that *P. fluorescens* (PGPR-1) strain possessed characters like tri calcium phosphate solubilization and ammonification which resulted in increased growth parameters including yield. Paul (2004) reported that *Pseudomonas* strains solubilized complex forms of 'P' in the soil, thus making it available to the plant. The intake of other minerals such as 'N' and 'P' was also found to be more with *P. fluorescens* treated black pepper plants. Samanta and Dutta (2004) elucidated that from among the six fluorescent rhizobacterial isolates tested, only four showed phosphate dissolution zones on Pikovaskya's TCP medium plates whereas the other two cultures, Pf W1 and P-2 showed phosphate solubilization in liquid medium. Bhatia *et al.* (2005) repoted that phosphate solubilization by the bacterial strains was found positive for six strains from among the ten which was confirmed with a clear zone on Pikovaskya's solid medium. Similarly, Parthasarathy (2005) reported the phosphate solubilizing efficacy of *P.fluorescens* in suppressing *Phytophthora* rot of black pepper.

Cakmakcj *et al.* (2007) studied the efficacy of different PGPR bacterial isolates to fix nitrogen and to solubilize phosphorus in barley for growth enhancement under greenhouse conditions in Turkey. They treated the barley seed with five different $N_2$ fixing bacteria *viz.*, *Bacillus licheniformis* RC02, *Rhodobacter capsulatus* RC04, *Paenibacillus polymyxa* RC05, *Pseudomonas putida* RC06 and *Bacillus* OSU-142 and with two phosphate solubilizing bacteria viz., *Bacillus megaterium* RC01 and *Bacillus* M-13 and compared with control and mineral fertiliser (N and P) application. The study revealed that, six PGPR isolates stimulated IAA production and three of them stimulated phosphate solubilization and all bacterial strains fixed $N_2$ and significantly increased the growth of barley.

Keyeo *et al.* (2011) studied the nitrogen fixing power of four diazotrophic bacteria *viz.*, *Azospirillum brasilense* (Sp7), *Herbaspirillum seropedicae* (Z78), *Enterobacter* sp. (L2) and *Gluconacetobacter* sp. (L15) in rice variety MR220 under green house conditions and found that all four strains were able to fix nitrogen with the same ability but produced phytohormone indole-3-acetic acid (IAA) in different concentration. Djuric *et al.* (2011) isolated 268

rhizozphere bacteria from maize in Serbia. Among them, one isolate PS2 showed abundant production of IAA (14 to 37 mM), siderophores, phosphate solubilization and growth inhibition of seven phytopathogenic fungi of different medicinal plants in Serbia. Shankarrao (2012) isolated 28 phosphate solubilising bacteria from rhizospheric soils of Neem, Mango and Jatropha plants. The solubilization index of each isolates was determined on Pikovskaya agar medium. The study revealed that the isolate M (III), M (III) col-2, M (III) col-4, N (b) col-1, N (c) col-2, J(A) and JC col-2 showed high P solubilization potential having SI=2.11-3.35 and quantitatively solubilized 160, 182, 270, 164, 200, 228 and 182 mg/ml P respectively after seven days incubation.

# Materials and Methods

## 3. MATERIALS AND METHODS

The present study entitled, 'Pathogenic and genetic variability in *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* and the management of bacterial blight disease' was carried out at Regional Agricultural Research Station (RARS), Pattambi and Departments of Plant Pathology and Agricultural Microbiology at College of Horticulture, Vellanikkara, Thrissur, Kerala, India during the period from September 2007 to August 2011.The experimental materials and the methodologies of the study are given below:

### 3.1. Survey on the occurrence of bacterial blight disease in three districts of Kerala

A survey was conducted during September 2007 in rice growing areas of Alappuzha, Palakkad and Thrissur districts of Kerala to study the occurrence of bacterial blight (BB) disease. Three locations in Alappuzha district *viz.*, Edathua, Karuvatta and Moncumbu, eight locations in Palakkad district *viz.*, Athimani, Erattakulam, Kodallur, Parali, Pattambi, Manchira, Nenmara and Polpully and three locations in Thrissur district *viz.*, Akamala, Kodakara and Mannuthy were selected for the study. In each location, five paddy fields were selected and four plots in each field having an average area of one square metre were selected at random. For assessing the per cent disease incidence (PDI), the number of infected plants and total number of plants were recorded. The PDI was calculated using the formula,

$$PDI = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} X\,100$$

From each plot, five plants were labeled and disease reactions were scored based on the Standard Evaluation Systems (SES) of rice (IRRI, 1996) as detailed below:

| Sl. No. | Description | Grade/ scale |
|---|---|---|
| 1 | 1 - 5% diseased leaf area | 1 |
| 2 | 6-12% diseased leaf area | 3 |
| 3 | 13-25% diseased leaf area | 5 |
| 4 | 26% - 50% diseased leaf area | 7 |
| 5 | 51% - 100% diseased leaf area | 9 |

Per cent disease severity (PDS) was calculated using the formula suggested by Wheeler (1969).

$$PDS = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves observed X Maximum disease grade}} X\,100$$

From PDI and PDS, Coefficient of Infection (CI value) was calculated

$$CI = \frac{PDI \times PDS}{100}$$

Based on the CI values, the disease reaction was categorized as described below:

| CI value | Category |
|---|---|
| 0-9 | Resistant (R) |
| 9.1-19 | Moderately resistant (MR) |
| 19.1-39 | Moderately susceptible (MS) |
| 39.1-69 | Susceptible (S) |

### 3.2. Collection, isolation, naming of different isolates of *X.oryzae* pv. *oryzae* (*Xoo*) and testing their pathogenicity

Diseased leaves showing water soaked lesions were cut into small pieces, surface sterilized with one per cent sodium hypochlorite solution for one minute and rinsed twice in sterile distilled water. The diseased leaf bits were washed in 70 per cent ethanol for one minute and rinsed thoroughly in sterile distilled water. Excess water was removed by placing the leaf bits on sterile blotting paper. The leaf bits were ground well with sterile water using pestle and mortar. A loopful of the suspension was streaked on to Peptone Sucrose Agar (PSA - Appendix I) plate and incubated at room temperature (28°C ±2°C) for two to four days. The plates were observed for the appearance of characteristic colonies of *Xoo*. The cultures were purified by repeated streaking on PSA medium, transferred to slants of the same medium and preserved at 4°C. The isolates were also preserved by taking a loopful of bacteria from a freshly streaked agar plate and suspended in

sterile tap water in eppendorf tubes and were stored at room temperature (28°C ±2°C) and at refrigerated conditions (4°C) for subsequent use without loss of virulence.

Isolates were assigned a code number like XAMI (1-5) where 'X' denotes *Xoo*, AMI refers to the location Athimani from where it was collected and 1-5 refers to the serial number of isolates obtained. Likewise other 13 isolates were also named after the location and the details were recorded.

The pathogenicity of the isolates was proved by inoculating on healthy rice seedlings of a highly susceptible variety Jyothi (PTB-39) at maximum tillering stage. Artificial inoculation was done by clip inoculation technique using 48 h old aqueous suspension of the different isolates grown on PSA medium. The inoculated plants were covered with polythene bags and high humidity was maintained by sprinkling water twice a day inside the polythene bags to initiate infection. Observations were recorded upto seven to ten days for the development of typical symptoms of the disease. The pathogens were reisolated from the artificially inoculated plants and compared with the original isolates.

### 3.3. Symptomatology

#### 3.3.1. Symptomatology under natural condition

Symptoms developed under natural condition on young seedlings (kresek phase) on 21 days old plants and symptoms at late tillering stage (leaf blight phase) on 65-70 days old plants were recorded during the survey at various locations.

#### 3.3.2. Symptomatology under artificial condition

To study the symptomatology of kresek phase under artificial condition, the different isolates were treated separately with seeds of susceptible variety Jyothi (PTB-39) and the symptoms developed on young seedlings were recorded. Similarly for studying the symptomatology of leaf blight phase, the different isolates were clip inoculated at the time of maximum tillering stage.

### 3.4. Studies on the cultural, morphological and biochemical characters of different isolates of *Xoo*

The isolates were subjected to various cultural, morphological and biochemical characters *viz*., colony characters, pigment production, Gram's reaction, starch hydrolysis, levan production, citrate utilization, gelatin liquefaction, arginine dihydrolase, $H_2S$ production, Voges-Proskauer test, Methyl red test, solubility in 3 per cent KOH, nitrate utilization, urease, oxidase and carbohydrate utilization tests *viz*., lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose and mannose following the methods as suggested in the Manual of Microbiological Methods, published by the Society of American Bacteriologists (1957) for appropriate identification as well as using the Laboratory Guide for Identification of Plant Pathogenic Bacteria (Schaad,1992).

### 3.5. Studies on pathogenic variability of *Xoo*

#### 3.5.1. Studies on leaf blight reaction on rice varieties/ near isogenic lines/differentials

The 20 commonly cultivated rice varieties of Kerala selected to study the pathogenic variability are given below:

| Sl.No. | Variety | Parentage |
|---|---|---|
| 1. | Aiswarya (PTB-52) | Jyothi x BR-51 |
| 2. | Anashwara (PTB-58) | Mutant of PTB-20 |
| 3. | Annapoorna (PTB-35) | Taichung Native T(N) -1 x PTB-10 |
| 4. | Aswathi (PTB-37) | PTB 10 x Dee--Gee-Woo-Gen |
| 5. | Bhadra (MO-4) | IR-8 x PTB-20 |
| 6. | Harsha (PTB-55) | PTB-10 x PTB-28 |
| 7. | Jaya | T(N)-1 x T-141 |
| 8. | Jyothi (PTB-39) | PTB-10 x IR-8 |
| 9. | Kairali (PTB-49) | IR-36 x Jyothi |
| 10. | Kanchana (PTB-50) | IR-36x Pavizham |
| 11. | Karuna (PTB-54) | Co-25 x H-4 |

| | | |
|---|---|---|
| 12. | Kunju Kunju Varna (VK-1) | Selection from local Kunju Kunju |
| 13. | Makaram (KTR-2) | Bulk progeny selection from local Cherady |
| 14. | Manupriya | PK3355-5-1-4 x Bhadra |
| 15. | Neeraja (PTB-47) | IR-20 x IR-5 |
| 16. | Matta Triveni (PTB-45) | Annapoorna x PTB-15 |
| 17. | Rohini (PTB-36) | PTB-10 x IR-8 |
| 18. | Sabari (PTB-40) | Annapoorna x IR8/2 |
| 19. | Swetha (PTB-57) | IR-50 x C14-8 |
| 20. | Uma (MO-16) | MO-6 x Pokkali |

The six near isogenic lines and three rice differentials obtained from the Directorate of Rice Research, Hyderabad, India were also subjected to study the pathogenic variability.

| **Near isogenic lines** | | **Rice differentials** |
|---|---|---|
| 1.IRBB 4(*Xa4*) | 4. IRBB 21(*Xa21*) | 1.Ajaya(*xa5/xaAj*) |
| 2. IRBB 5(xa5) | 5. IRBB 57(*Xa4/xa5/Xa21*) | 2.IR 8 |
| 3.IRBB 3(x*a13*) | 6. IRBB60(*Xa4/xa5/xa13/Xa21*) | 3.IR 24 |

Cement troughs of 6x1.5M$^2$ constructed inside the net house were used for conducting the experiment. Twenty one days old seedlings were transplanted at the rate of two seedlings/hill with a spacing of 15 cm x10 cm. The inoculum was prepared by suspending 48 h old cultures of *Xoo* from PSA medium in sterile water. Concentration of the bacterial suspension was adjusted to $10^8$cfu/ml. Inoculation was done on 40 days old rice plants using sterile scissors dipped in the bacterial suspension (Kauffmann *et al.*, 1973). The scissors used for the inoculation of different isolates were sterilized each time by dipping in alcohol. The control plants were maintained by inoculating with sterile water.

Disease reactions were scored at 21 days after inoculation (DAI). For this, PDI, PDS and CI values were assessed as mentioned under 3.1.

Similarly, the lesion length on the leaves was recorded at 21 DAI and the disease reaction was categorized as described by Akhtar *et al.* (2008).

| Lesion length (cm) | Category |
|---|---|
| 1-5 | Resistant (R) |
| 5-10 | Moderately resistant (MR) |
| 10-15 | Moderately susceptible (MS) |
| 15 to above | Susceptible (S) |

Observations on the time taken for initiation of symptom (incubation period), initial and final symptom expression were recorded at 21 DAI.

### 3.5.2. Studies on kresek symptom on rice varieties

Rice seeds (10g) of 20 varieties were treated with bacterial suspension ($10^8$cfu/ml) of 14 isolates of *Xoo* in plastic cups separately for 30 minutes. The variety Makaram was substituted with Bharathi. Five hills in a row, consisting two seed per hill from each rice variety were sown with a spacing of 15 cm x10 cm. Scoring of kresek disease incidence was done on 21 days old plants as mentioned in 3.1.

### 3.5.3. Classification of different isolates of *Xoo* based on disease reaction

The 14 isolates were grouped as highly virulent, moderately virulent and weakly virulent based on the field survey, colony characters and leaf blight reaction on cultivated varieties and on near isogenic lines/rice differentials.

## 3.6. Study on genetic variability of *Xoo* isolates from three districts of Kerala

### 3.6.1. Isolation of genomic DNA

The genomic DNA from 14 isolates of *Xoo* obtained from three rice growing districts of Alappuzha, Palakkad and Thrissur were extracted using the standard protocol of hexadecyl-trimethyl ammonium bromide (CTAB) method suggested by Melody (1997) with slight modifications. Actively grown culture of 25 ml each of the isolates was centrifuged at 6,000 rpm for 5 min at 4°C and the supernatant was removed. The pellet was suspended in 1 ml TE buffer,

added with 0.5 ml of 1-butanol, vortexed well to mix with the cells to remove extracellular materials and centrifuged at 5000 rpm for 5 min at 4°C. The supernatant was discarded and the pellet was re suspended in 2 ml of TE buffer and centrifuged again to remove all traces of butanol. Again the pellet was re suspended in 1 ml TE buffer, added with 100 µl lysozyme (10 mg $ml^{-1}$ freshly prepared) and incubated at room temperature (28°C ±2°C) for 5 min. After incubation, 100 µl of 10 per cent SDS and 25 µL of 100 µg $ml^{-1}$ proteinase K were added, mixed well and incubated at 37°C for 1 h. To this, 200 µl of 5 M of NaCl was added and mixed well. Then 150 µl of CTAB (Annexure II) was added, mixed well and incubated at 65°C for 10 min. The mixture was extracted with 1 ml of phenol: chloroform mixture, mixed well and centrifuged at 6000 rpm for 15 min at 4°C. The aqueous layer was transferred carefully to a 2.0 ml microfuge tube and DNA was precipitated by adding 0.6 volume of ice cold isopropanol, incubated from 1 h to overnight at -20°C. The DNA was pelletized by centrifugation at 12000 rpm for 15 min at 4°C. The pellet was washed with 70 per cent ethanol, dried *in vacuo* for 10 min and re suspended in 50 µl of TE buffer. One µl DNAse free RNAse (10 mg/ml) was also added by swirling and incubated at 37°C for 30 min and the DNA was stored at -20°C for further use.

**BOX and ERIC-PCR fingerprinting**

| Primer | Sequence | References |
|---|---|---|
| BOX A1R | 5'-CTACGGCAAGGCGACGCTGACG-3' | Versalovic *et al.* (1994) |
| ERIC 1R | 5'-ATGTAAGCTCCTGGGGATTCAC-3' | Versalovic *et al.* (1991) |
| ERIC 2 | 5'-AAGTAAGTGACTGGGGTGAGCG-3' | Versalovic *et al.* (1991) |

**PCR mixture**

BOX and ERIC-PCR mixtures (25µl each) for all the isolates were prepared using the following recipe:

**Master mix**

| Quantity | Stock solution |
|---|---|
| 2.50 µl | 10 x Taq Buffer |
| 0.20 µl | BSA, 20 mg/ml |
| 2.50 µl | DMSO, 100% |
| 15.15 µl | $ddH_2O$, use 15.65 µl for BOX |
| 1.25 µl | Mix of dNTP's (1:1:1:1) 100 mM each |
| 1.00 µl | Primer 1 (0.3 µg/µl ) |
| 1.00 µl | Primer 2 , not applicable for BOX (0.3 µg/µl ) |
| 0.40 µl | Taq DNA Polymerase, 5 U/µl |

**PCR programming**

PCR programming was done using the above mixtures with 1 µl of DNA from each isolate (genomic DNA–approximately 50-100 ng/µl). They were mixed well by brief centrifugation and the PCR programming was done as mentioned below:

Initial denaturation : 95°C for 7 minutes followed by 30 cycles

| BOX | ERIC |
|---|---|
| Denaturation 94°C for 1 min. | Denaturation 94°C for 1 min. |
| Annealing 53°C for 1 min. | Annealing 52°C for 1 min. |
| Extension 65°C for 8 min. | Extension 65°C for 8 min. |

Final extension : 65°C for 16 min

**3.6.2. Agarose gel electrophoresis**

Agarose gel electrophoresis was performed to check the quality of DNA and also to separate the products amplified through polymerase chain reaction. Then 1X TAE (Annexure II)

buffer in 500 ml quantity was prepared to fill the electrophoresis tank and to prepare the gel. In a separate conical flask, agarose (1.5%) was added to 1X TAE buffer; boiled till the agarose dissolved completely and cooled to lukewarm temperature. Ethidium bromide (50 mg/ml stock) was added at the rate of 5 µl per 100 ml of agarose solution and was allowed to mix completely. It was then poured into the gel mould; the comb was placed properly and allowed to solidify for half an hour at room temperature (28°C ±2°C). After solidification, the comb was removed carefully. The caste gel was placed in the electrophoresis tank containing 1X TAE buffer with the well near the cathode and submerged to a depth of 1 cm. Then 15 µl of the PCR product was mixed with 3 µl of 10X tracking dye (Annexure II) and mixed well by pipetting in and out for three times. The mixture was loaded into the wells with the help of micropipette. Six µl of ready-to-use 1 kb DNA ladder (Fermentas, USA) (500 ng of DNA/lane) was loaded in one of the wells as a standard marker. The cathode and anode were connected to power pack using power cord and the gel was run at a constant voltage of 60 volts. The negatively charged DNA molecules move towards the anode and get separated according to their molecular weight. The power was turned off when the tracking dye migrated appropriate distance in the gel and the gel was viewed under UV transilluminator and the banding pattern was analyzed. All the gels viewed were documented in the form of photographs using Alpha Imager TM1200 documentation and analysis system.

### 3.6.3. Dendrogram

Banding patterns of BOX and ERIC-PCR products in 1.5 per cent agarose gel electrophoresis were visualized by ethidium bromide staining and documented in Alpha Imager TM1200 documentation and analysis system. In order to determine the similarity between the isolates, a binary matrix was established recording the presence or absence of bands. UPGMA algorithm was used for hierarchical cluster analysis. Pair-wise comparisons were calculated using Jaccard's coefficient (Jaccard, 1912) and dendrogram was built using the UPGMA method (Nei and Li, 1979) using NTSYS-PC2 package (Numerical taxonomy analysis program package, External software, USA).

### 3.7. Management of bacterial blight of rice

As the first step to manage the bacterial blight pathogen, the efficacy of selected antibiotics, organics and agrochemicals were tested under *in vitro*. The bacteria from rhizosphere, endosphere, cow dung and vermicompost were isolated and tested for their antagonism against *Xoo*.

#### 3.7.1. *In vitro* sensitivity of bactericides to highly virulent isolates of *Xoo*

*In vitro* sensitivity of different bactericides was studied against highly virulent isolates of the pathogen *viz.*, Athimani (XAMI-3), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) of Palakkad district. The details of the treatments are given below:

| Sl. No. | Antibiotics | Conc.(ppm) |
|---|---|---|
| 1 | Tetracycline | 50,100, 250 |
| 2 | Streptocycline | 50, 100, 200, 250 |
| 3 | Streptomycin sulphate | 50,100, 250 |
| 4 | Bactrinashak | 50,100, 250 |
| 5 | Ampicillin | 50,100, 250 |
| 6 | Penicillin | 50,100, 250 |

Sterile paper discs of 6 mm diameter dipped in selected concentrations were aseptically placed at the centre of Petri dishes containing the PSA medium pre seeded with 48 h old virulent isolates of the pathogen. Five replications were maintained for each treatment. Observations on the zone of inhibition were recorded after 48 h of incubation at room temperature (28°C ±2°C).

#### 3.7.2. *In vitro* sensitivity of organics and agrochemicals to highly virulent isolate of *Xoo*

*In vitro* sensitivity of different organics and agrochemicals against the highly virulent isolate of Polpully (XPLY-5) of *Xoo* was studied. The details of the treatments are given below:

| | |
|---|---|
| T1. Cow dung extract 2% | T6. Copper oxychloride 500 ppm |
| T2. Vermicompost extract 2% | T7. Copper hydroxide 0.15% |
| T3.Cow dung extract 2% +Vermicompost extract 2% | T8. Copper oxychloride 500 ppm + Streptocycline 150 ppm |
| T4.Cow dung extract 2% + *Pseudomonas fluorescens* 2% | T9. *P. fluorescens* 2% |
| T5. Vermicompost extract 2% + *P. fluorescens* 2% | |

Sterile paper discs of 6 mm diameter dipped in selected concentrations of different organics and agrochemicals were aseptically placed at the centre of Petri dishes containing the PSA medium pre seeded with 48 h old virulent Polpully isolate (XPLY-5) of the pathogen. Five replications were maintained for each treatment. Observations on the zone of inhibition were recorded after 48 h of incubation at room temperature (28°C ±2°C).

### 3.7.3. Enumeration of total microflora from rhizosphere and endosphere of rice and isolation of antagonistic bacteria

Rhizosphere soil samples from major rice growing tracts of Alappuzha, Palakkad, and Thrissur districts were collected during December – January 2008. A total of 56 soil samples (four samples per location) from rice fields of Edathua, Karuvatta and Moncumbu of Alappuzha district; Akamala, Kodakara and Mannuthy of Thrissur district; and Athimani, Erattakulam, Kodallur, Parali, Pattambi, Manchira, Nenmara and Polpully of Palakkad district were collected. Similarly 56 healthy rice plant samples (four plants per location) adjacent to the infected plants were also collected for the isolation of bacterial endophytes.

The samples were pooled separately, shade dried and the total microflora were quantitatively estimated by serial dilution plate technique (Johnson and Curl, 1972). Martin's Rose Bengal Streptomycin Agar, Thornton's standardized Agar and Ken Knight's Agar media (Appendix I) were used for estimating the total microflora *viz.*, fungi, bacteria and actinomycetes at dilutions of $10^{-4}$, $10^{-7}$ and $10^{-5}$ respectively. Representative rhizobacterial colonies from the medium used for enumeration of bacteria were selected, purified and maintained for further studies.

In addition, isolation of rhizobacteria using Nutrient agar (NA) and Kings'B (KB) for isolation of *Pseudomonas* spp., Soil Extract agar (SEA), Methyl Red agar (MRA) for isolation of

Gram positive bacteria and Crystal Violet agar (CVA) for isolation of Gram negative bacteria (Appendix I) were also carried out for selecting appropriate bacterial isolates. Typical bacterial colonies developed in the dilution plates from each medium were picked up and purified. Altogether 70 rhizobacterial isolates were selected and the cultures were maintained on NA slants by sub culturing at fortnightly intervals and also in sterile water and preserved at 4°C for further use.

For the isolation of total endosphere microflora, whole rice plants at tillering stage were uprooted and brought to the laboratory. After washing the plants under running tap water, small bits of stem from the collar region and young roots were taken separately using sterilized knife. The stem and root sections of 1000 mg were weighed separately and surface sterilized with two per cent sodium hypochlorite solution for 10 minutes and rinsed four times with sterile 0.02 M potassium phosphate buffer (PB). An aliquot of 0.1 ml was taken from the final wash and spread over the solidified Nutrient agar and PDA mediated in sterile Petri dishes which served as sterility check. Samples were discarded if growth was detected in the sterility check within 48 h. Each sample (1000 mg) was triturated with a sterile mortar and pestle in 9.0 ml of the potassium phosphate buffer. From this, serial dilutions up to $10^{-7}$ of the triturate were made in the buffer. From $10^{-4}$ dilution, 0.1 ml each from stem and root was transferred to Petri dishes with Martin's Rose Bengal Streptomycin Agar for fungi; from $10^{-7}$ dilution, 0.1 ml each from stem and root was transferred to Petri dishes with NA medium for bacteria and $10^{-5}$ dilution; 0.1 ml each from stem and root was transferred to Petri dishes with Ken Knight's agar for actinomycetes. Three replications were maintained for each dilution. The plates were incubated at room temperature (28°C ±2°C) and observed for the growth of endophytes from next day onwards. From these, 20 endobacterial colonies were transferred to slants of nutrient agar and cultures of these endophytes were maintained as pure cultures by sub culturing for further work.

### 3.7.3.1. Enumeration of total microflora from cow dung and vermicompost and isolation of antagonistic bacteria

The total microflora were isolated from 10 samples each from cow dung and vermicompost collected from Pattambi area of Palakkad district using the serial dilution technique as mentioned in 3.7.3. A total of 20 bacterial colonies were isolated and maintained on NA slants.

**3.7.3.2. *In vitro* evaluation of antagonistic property of bacterial isolates against *Xoo***

The *in vitro* antagonistic effect of 110 bacterial isolates obtained from rhizosphere, endophere, cow dung and vermicompost against the highly virulent isolate of *Xoo* from Polpully (XPLY-5) was tested by dual culture method (Dennis and Webster,1971). For preliminary screening, NA seeded with 48 h old culture of the pathogen in Petri dish was spot inoculated with the bacterial isolates. In each plate, four different bacterial isolates were spot inoculated at equidistant points and 2 cm away from the periphery of the plate. The plates were incubated at room temperature (28°C ±2°C) and observed for zone of inhibition of the pathogen after 48 h. The plates with pathogen alone served as control.

Out of the 110 bacterial isolates tested in preliminary screening, only 18 bacterial isolates showed the antagonism against *Xoo* and the remaining isolates have over grown on the pathogen. The six isolates which showed prominent inhibition zone were selected for further studies. These six antagonists were spot inoculated at the centre of the NA plates seeded with *Xoo*. Three replications were maintained for each antagonist. The plates with pathogen alone served as control. The reference culture of *Pseudomonas fluorescens* (KAU-Pf1) served as standard check. The plates were incubated at room temperature (28°C ±2°C) and observed for inhibition of the pathogen after 48 h and the per cent inhibition was calculated using the formula (Vincent,1927).

$$PI = \frac{C - T}{C} \times 100 \quad \text{where,}$$

PI - Per cent Inhibition.

C - Growth of the pathogen in control plates (cm)

T - Growth of the pathogen in dual culture (cm)

The antagonism index was calculated using the formula,

$$AI = PI \times IZ$$

where, AI – Antagonism index

PI – Per cent inhibition

IZ – Inhibition zone (cm)

The inhibition zone (IZ) produced by each isolate was further scored following the scale as: IZ : 1 cm = 1; 1-2 cm = 2; 2-3cm = 3; > 3cm = 4

The details of the bacterial antagonists selected are given below:

| Sl.No. | Isolate No.* | Location |
|---|---|---|
| 1 | RR-26 | Nenmara |
| 2 | RR-53 | Pattambi |
| 3 | RE-1 | Kodallur |
| 4 | CB-39 | Pattambi |
| 5 | VB-67 | Pattambi |
| 6 | VB-69 | Pattambi |

* RR -Rice Rhizosphere Bacteria * RE - Rice Endosphere Bacteria

* CB – Cow dung Bacteria * VB -Vermicompost Bacteria

### 3.8. Cultural, morphological and biochemical characterization of promising antagonistic bacteria

Cultural characters of the antagonistic bacteria were studied on the NA medium. The cultures were streaked on NA medium in Petri dishes and incubated at room temperature and the colonies were observed for shape, elevation and margin. For morphological studies, 24 h old cultures of the bacteria were used. Gram staining was done to study the Gram's reaction. Shape of the bacteria was observed under oil immersion objective of the microscope. To confirm the results of Gram's reaction, KOH test was conducted. Biochemical characterization of the six promising bacterial antagonists *viz.*, RE-1, RR-26, CB-39, RR-53, VB-67 and VB-69 was carried out following the methods as suggested in the Manual of Microbiological Methods, published by the Society of American Bacteriologists (1957) and also by the Bergey's Manual of Systematic Bacteriology, Vol I (Staley *et al.*, 1989). The biochemical characters *viz.*, Gram's reaction, starch hydrolysis, levan production, gelatin liquefaction, arginine dihydrolase, Voges-Proskaeur test, Methyl red test, solubility in 3% KOH, oxidase and urease tests were conducted. The Hi Assorted TM Biochemical Test kit for Gram negative rods was also employed for characterization of citrate utilization, lysine decarboxylase, ornithine decarboxylase, urease, deamination, nitrate reduction, $H_2S$ production, glucose, adonitol, lactose, arabinose and sorbitol tests by pippeting out 50 µl of bacterial suspension in each well and observed for the colour change after 24 h.

### 3.9. *In planta* evaluation of antagonists, organics and agrochemicals against *Xoo*

A pot culture experiment was laid for the evaluation of selected antagonists, antibiotics, fungicides and other organics *viz.*, cow dung extract and vermicompost extract against the virulent Polpully isolate of *Xoo*. The details of experiment are given below:

| | |
|---|---|
| Variety | : Jyothi (PTB -39) |
| Design | : CRD |
| Replications | : 5 |
| No. of plants/pot | : 3 |
| No. of plants/treatment | :15 |
| Treatments | : 21 |

Treatment details:

| | |
|---|---|
| T1. Rice Rhizosphere Bacteria (RR-26) | T12. Bactrinashak 250 ppm |
| T2. Rice Rhizosphere Bacteria (RR-53) | T13. Cow dung extract 2 % |
| T3. Rice Endosphere Bacteria (RE-1) | T14. Vermicompost extract 2% |
| T4. Cow dung Bacteria (CB-39) | T15. Cow dung extract 2%+ Vermicompost extract 2% |
| T5. Vermicompost Bacteria (VB-67) | T16. Cow dung extract 2% + *P. fluorescens* 2% |
| T6. Vermicompost Bacteria (VB-69) | T17. Vermicompost extract 2% + *P. fluorescens* 2% |
| T7. Tetracycline 50 ppm | T18. Copper oxychloride 500 ppm |
| T8. Tetracycline 100 ppm | T19. Copper hydroxide (0.15%) |
| T9. Tetracycline 250 ppm | T20. KAU-Pf1 (*P. fluorescens* 2% reference culture) |
| T10. Streptocycline 200 ppm | T21. Absolute control |
| T11. Streptocycline 250 ppm | |

#### 3.9.1. Preparation of potting mixture

Earthen pots of size 10"x 10" were filled with soil from paddy field containing soil and cow dung at the ratio of 3:1.

### 3.9.2. Preparation of bacterial inoculum for application

The selected six bacterial antagonists and KAU-Pf1 (*P. fluorescens* - reference culture) were grown separately on nutrient agar for 48 h. The bacterial cells were harvested by scraping the lawn with sterile microscopic slide and suspended in luke warm water containing carboxy methyl cellulose sodium salt as sticker @ 0.5%. Then concentration was adjusted to $10^8$cfu/ml and used as inoculum for seed treatment, seedling root dip and foliar spray.

### 3.9.3. Seed treatment

Rice seeds were surface sterilized with one per cent sodium hypochlorite solution for one minute and washed with three changes of sterile distilled water. The seeds were treated with six promising bacterial antagonists, KAU-Pf1 and bactericides separately and were sown in nursery beds.

### 3.9.4. Seedling root dip and transplanting

When the seedlings were of 20 days old, they were uprooted, dipped in bacterial suspension of six promising antagonists and KAU- Pf1 (reference culture) and were transplanted to earthen pots containing soil from paddy field. Each treatment was replicated in five pots at the rate of three rice seedlings/pot. Seedlings without any treatments served as control.

### 3.9.5. Preparation of pathogen inoculum for inoculation

Highly virulent isolate of *Xoo* (Polpully-XPLY-5) (48 h old) was used for the inoculation of the rice plants at the time of maximum tillering stage. The concentration of the bacterial cells was adjusted to $10^8$cfu/ml.

### 3.9.6. Artificial inoculation of *Xoo*

All the treatments including control were inoculated with the pathogen at maximum tillering (27 DAT) by clip inoculation method.

### 3.9.7. Foliar spray

Foliar spray with selected bacterial antagonists, bacterial bio control agent (Pf1), antibiotics, fungicides and cowdung extract/vermicompost extract were given on third day after the clip inoculation of *Xoo* at 30 DAT and spraying repeated at 45 DAT.

## 3.10. Disease reaction due to various treatments

### 3.10.1. Assessment of per cent disease incidence (PDI), per cent disease severity (PDS) and coefficient of infection (CI)

Disease incidence and disease severity were scored and recorded as per the procedure given in 3.1. at 60 DAT and PDI, PDS and CI values were calculated to assess the disease reaction due to various treatments.

## 3.11. Effect of various treatments on the biometric characters of rice plants

### 3.11.1. Growth characters

#### 3.11.1.1. Plant height

Height of five plants in each treatment was measured from the base of the plant to the tip of the top leaf at 60 DAT. The mean height was computed and expressed in cm.

#### 3.11.1.2. Number of tillers

Number of productive tillers was counted from five hills in each treatment at 60 DAT. The mean number of tillers was computed and expressed as number $hill^{-1}$.

#### 3.11.1.3. Root length

Length of roots in five randomly selected and uprooted hills at the time of harvest were measured and the mean expressed in cm.

#### 3.11.1.4. Root dry weight

The roots from five randomly selected and uprooted hills were washed free of soil and separated from the stem. They were first air dried and then oven dried at 60°C to constant weight. The root dry weight was recorded and the mean expressed in g $hill^{-1}$.

### 3.11.2. Yield attributes

#### 3.11.2.1. Panicle length

Length of five randomly selected panicles was measured and the mean expressed in cm.

#### 3.11.2.2. Number of filled grains/panicle

No. of filled grains of five randomly selected panicles were calculated and mean was calculated and expressed as no. of filled grains/panicle.

#### 3.11.2.3. Thousand grain weight

One thousand grains were collected, at random, from the produce of each treatment and their weight was recorded in g.

#### 3.11.2.4. Yield/pot

The grains from each pot, after winnowing and cleaning, were weighed and grain yield was computed at 13 per cent moisture and expressed in g per pot.

#### 3.11.2.5. Straw yield

The straw from each pot was sun dried uniformly, weighed and expressed in g per pot.

### 3.12. Studies on the compatibility of bacterial antagonists with common agrochemicals against *Xoo*

The *in vitro* compatibility of bacterial antagonists, pesticides and fertilizers were studied against *Xoo* by paper disc method. All possible two way combinations were studied among themselves and with others. The details of antagonists, pesticides and fertilizers used for this study are listed below:

| Sl. No. | 1. Antagonists |
|---|---|
| 1. | RE-1 (Rice Endosphere bacteria) |
| 2. | RR-26 (Rice Rhizosphere bacteria) |
| 3. | RR-53 (Rice Rhizosphere bacteria) |
| 4. | CB-39 (Cow dung bacteria) |
| 5. | VB-67 (Vermicompost bacteria) |
| 6. | VB-69 (Vermicompost bacteria) |
| 7. | KAU-Pf1 (*Pseudomonas fluorescens*) |

| Sl. No. | 2. Pesticides | Trade name |
|---|---|---|
| 1. | Mancozeb | Indofil M-45 |
| 2. | Carbendazim | Bavistin-50 WP |
| 3. | Propiconazole | Tilt-25 EC |
| 4. | Hexaconazole | Contaf-5 EC |
| 5. | Chlorpyriphos | Dursban-50 EC |
| 6. | Dimethoate | Rogor-30 EC |
| 7. | Dichlorvos | Nuvan-76 WSC |
| 8. | Triazophos | Hostothion-40 EC |
| 9. | Quinalphos | Ekalux-25 EC |

| Sl. No. | 3. Fertilizers |
|---|---|
| 1. | Urea (N- 46 %) |
| 2. | Rajphos ($P_2O_5$ - 18-20%) |
| 3. | Muriate of Potash ($K_2O$- 60%) |
| 4. | Ammonium sulphate (N- 20.50%) |

Concentration of different antagonistic bacteria was adjusted to $10^8$ cfu/ml. To prepare correct concentration of agrochemicals as per the Packages of Practices recommendations (POP) of Kerala Agricultural University, the actual quantity was mixed with 100 ml of sterile water and the stock solutions were made. Fertilizers were exposed to UV light for 45 minutes to avoid the contamination.

Three discs were kept in each Petri dish dipped in two materials for studying the combination effect and the interaction between them. The discs dipped in single material were used to study the individual effect and they served as control also. The studies were carried out on PSA medium seeded with *Xoo*. Observations on zone of inhibition of *Xoo* were recorded after 48 h of incubation.

### 3.13. Mode of action of promising bacterial antagonists

Six bacterial isolates along with Pf1 (reference culture) were selected for the various mechanisms involved in the antagonistic reaction against *Xoo* under lab condition by the following methods:

#### 3.13.1. Production of hydrogen cyanide

Production of hydrogen cyanide (HCN) by the antagonistic bacterial isolates was detected by following the method of Wei *et al.* (1991). Log phase of bacterial culture (25 µl) was inoculated to 25 ml of Kings'B broth supplemented with 4.4 g $l^{-1}$ of glycine taken in a sterile Petri plate. Sterile filter paper strips soaked in picric acid solution (2.5 g picric acid + 12.5 g $Na_2CO_3$ in 1000 ml of water) were placed in the lid of each plate. Petri dishes were sealed with parafilm and incubated for 72 h in a slow shaking platform. Change in colour of filter paper strips from yellow to brown and to red indicated the production of hydrogen cyanide. The reaction was assessed based on a 1- 4 scale depending on the colour gradation.

#### 3.13.2. Production of ammonia

The qualitative estimation of ammonia production was done following the method of Dye (1962). The selected antagonistic bacterial cultures were grown in 25 ml of peptone water (Appendix I) and incubated at 30°C for four days. Three replications were maintained for each isolate. After incubation, 1 ml of Nessler's reagent was added to the broth. The presence of faint yellow to deep yellow or brown colour indicated production of ammonia. The reaction was scored as nil, low, medium and high in 1-4 scale based on the intensity of colour.

### 3.13.3. Phosphorous (P) solubilization

The phosphate solubilizing capacity of the potential bacterial isolates was tested *in vitro* using Pikovskaya's agar (Appendix I) as well as in its broth (Pikovskaya, 1948). Then 10 µl of log phase of the bacterial isolates were spot inoculated at the centre of the plate containing the medium and incubated at 28°C for five days. Plates were observed for the appearance of clear zone around the colony and its diameter was measured. Three replications were maintained for each isolate.

For quantitative estimation of phosphorus solubilization, the bacterial isolates were inoculated to 25 ml of Pikovskaya's broth and incubated for 48 h at 28°C at 150 rpm in an orbital shaking incubator. Medium without inoculation served as control. The cultures were centrifuged at 7000 rpm for 10 min at 4°C. Supernatant was collected and pellet discarded. One ml of supernatant was taken in a test tube and diluted by adding 6 ml of distilled water. Then 2 ml of chloromolybdic acid (15g of ammonium molybdate in 400 ml of warm distilled water, added 342 ml of 12 N HCl, cooled and made upto one litre) and one ml of chlorostannous acid (2.5 g of the $SnCl_2$. $2H_2O$ in 10 ml of conc. HCl, heated gently and volume made upto 100 ml after cooling) were added to the mixture. Absorbance of the mixture was read at 660 nm using a spectrophotometer (Spectronic-20 D+). From the standard graph (for 100 ppm 'P' solution, 0.439 g of dried $KH_2PO_4$ in 400ml of distilled water and to this added 25 ml of 7 N $H_2SO_4$ and made upto one litre), amount of phosphate released from tricalcium phosphate by bacteria were calculated. The 'P' solubilization capacity of the isolates was also scored following the scale based on the 'P' solubilization as:
$>1<3$ mg $50ml^{-1} = 1$; $>3<6$ mg$50ml^{-1} = 2$; $>6<9$ mg $50ml^{-1} = 3$; $> 9$ mg $50ml^{-1} = 4$

### 3.13.4. Assay of growth promoting hormone - Indole Acetic Acid (IAA)

A modified protocol suggested by Bric *et al.* (1991) was used to estimate the IAA production by selected bacterial isolates. A loopful of the bacterial culture was inoculated in 25 ml broth of Luria - Bertani medium (LB) (Appendix I) amended with 100 µg$ml^{-1}$ of tryptophan (100 µg$ml^{-1}$ tryptophan in 50 % ethanol) as precursor and incubated on a rotary shaker for 30 h. The supernatant from the bacterial isolates were collected after centrifugation at 10,000 rpm for 10 minutes. To these 1 ml cell free culture filtrate (CFCF), two drops of o-phosphoric acid and 2 ml of Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35 per cent $HClO_4$) were added and incubated at 28°C for 30 min and the absorbance was measured at 530 nm (Spectronic-20 D+). A

standard curve was prepared with different concentrations of IAA and was used to quantify the IAA production and these were finally scored in a scale as:

$>0<15\ \mu g ml^{-1}=1;\quad >16<30\ \mu g ml^{-1}=2;\quad >31<45\ \mu g ml^{-1}=3;\quad >46\ \mu g ml^{-1}=4$

**3.13.5. Detection of siderophores**

The promising bacterial isolates along with the reference culture (Pf1) were tested for the production of iron-chelating siderophores.

**3.13.5.1. Detection of siderophores by UV fluorescence method**

Log phase of the rhizobacterial isolates including the reference culture of KAU-(Pf1) were streaked on to a Kings'B plate and incubated at 28°C for 48h. The plates were observed on a UV transilluminator to view the fluorescence (Kloepper *et al.*, 1980b).

**3.13.5.2. Detection of siderophores by Chrome azurol (CAS) assay**

Siderophore production by the bacterial isolates was determined by CAS assay as described by Vellore (2001). The bacterial isolates were grown in modified Fiss minimal medium (Appendix I) containing 0.5 µM added iron for 24 h on a rotary shaker at 27°C. For this, all glasswares used to store the stock solutions of the modified Fiss minimal medium were treated with concentrated hydrochloric acid (HCl) and then rinsed with Milli pore water, to remove traces of contaminating iron.

The CAS plates were employed to check the culture supernatant for the presence of siderophore. The CAS plates were prepared in three separate steps.

The first step was the preparation of CAS indicator solution. For this, initially, 60.5 mg of chrome azurol S was dissolved in 50 ml of Millipore water. Then 10 ml of Fe III solution (27 mg $FeCl_3.6H_2O$ and 83.3µl concentrated HCl in 100ml Milli pore water) was added, along with 72.9 mg hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 ml Millipore water.

The HDTMA solution was added slowly while stirring, resulting in a dark blue solution (100 ml total volume), which was then autoclaved.

The second step involved was preparation of basal agar medium. In a 250 ml flask, 3g 3-(N-Morpholino) propane sulfonic acid (MOPS) (0.1 M), 0.05g NaCl, 0.03 g $KH_2PO_4$, 0.01 g ammonium chloride ($NH_4Cl$) and 0.05 g L-Asparagine were dissolved in 83 ml Milli pore water. The pH of the solution was adjusted to 6.8 using 6 M NaOH. The total volume was brought to 88 ml using Millipore water, and 1.5 g agar was added to the solution while stirring and heating until melted. The solution was then autoclaved.

The third step was the preparation of CAS agar plates. Here, the autoclaved basal agar medium was cooled to 50°C in a water bath. The CAS indicator solution was also cooled to 50°C, along with 50 per cent solution of glucose. Once cooled, 2 ml of 50 per cent glucose solution was added to the basal agar medium with constant stirring, followed by addition of 10 ml of CAS indicator solution, which was added carefully and slowly along the walls of the flask with constant stirring, but at a speed so as not to generate any bubbles. Once mixed thoroughly, the resulting solution (100 ml) was poured into sterile plastic plates, each plate receiving approximately 25 ml of blue agar. Under minimal iron conditions, siderophore was produced and released into the culture medium. To isolate and collect siderophore, the antagonistic bacterial cultures were grown in iron-restricted (0.5 µM added iron) modified Fiss minimal medium with a high concentration of iron (20 µM). After 24 h of growth, the culture was centrifuged at 13,500 rpm for two minutes and the cell free culture filtrate (CFCF) was collected. A well was made on the CAS plate with 0.6 cm cork borer and 60µl of CFCF was added to the well and the plate was incubated at room temperature (28°C ±2°C). A maximum of 8 h was given for any colour change to develop. Siderophore was detected by the presence of an orange halo around the well. The control consisted of culture grown in high iron medium and uninoculated medium.

### 3.13.6. Production of non volatile metabolites

This test was carried out using cellophane paper method described by Dennis and Webster (1971). For this, cellophane paper (50 µm thick) discs of 90 mm diameter were taken and srerilized in an autoclave at 121°C for 15 minutes and then each sterilized disc was aseptically placed over PSA plates (90 mm diameter). Discs of 10 mm diameter were taken from lawn/growth of each

candidate antagonistic bacterial isolate and placed at the centre of the cellophane paper and incubated for 48-72h. After this, the cellophane paper along with the adhering antagonist was removed carefully and a 10 mm disc of pathogen was immediately placed on the medium at central position previously occupied by the candidate antagonist. The radial growth of the pathogen was recorded at 48 h interval up to three days and compared with its growth in control. Three replications were maintained and the per cent inhibition of the pathogen over control was calculated.

### 3.14. Field experiment for the management of bacterial blight disease of rice

A field experiment was laid out to study the antagonistic and growth promoting efficiency of six selected bacterial antagonists, one reference bacterial bio control agent (Pf1) along with one chemical, three antibiotics and two organics against the virulent Polpully isolate (XPLY-5) of *Xoo*. The details of experiment are given below:

| | |
|---|---|
| Variety | : Jyothi (PTB-39) |
| Design | : RBD |
| Replications | : 3 |
| Plot size | : 3m x 2m |
| Spacing | : 15cmx10cm |
| Treatments | :17 |

Treatment details :

T1. Bacterial antagonist (RR-26)
T2. Bacterial antagonist (RR-53)
T3. Bacterial antagonist (RE -1)
T4. Bacterial antagonist (CB -39)
T5. Bacterial antagonist (VB -69)
T6. Bacterial consortium (RE-1 + CB -39)
T7. Bacterial consortium (CB -39+VB -69)
T8. Streptocycline 50 ppm as per POP
T9. Streptocycline 250 ppm
T10. Bactrinashak 250 ppm
T11. Tetracycline 50 ppm
T12. Tetracycline 100 ppm
T13. Cow dung extract spray 2% as per POP
T14. Cow dung extract 2%+ Vermicompost extract 2%
T15. Cow dung extract 2% + KAU-(Pf1) 2%
T16. Pf1( reference culture ) 2%
T17. Absolute control

The treatments T1 to T7 and T16 were given as seed treatment, seedling root dip and foliar spray. The treatments T8 to T12 were given as seed treatment and foliar spray. The treatments T13 to T15 were given with foliar spray alone.

### 3.14.1. Preparation of field

Main field was prepared by puddling the soil amended with the cowdung @ 5t/ha and allowed for decomposition for 15 days and again the field was ploughed and leveled.

### 3.14.2. Preparation of bacterial inoculum

The selected five bacterial antagonists and one bacterial bio control agent (Pf1) were grown separately on nutrient agar for 48 h. The bacterial cells were harvested by scraping the lawn with sterile microscopic slide and suspended in luke warm water containing carboxy methyl cellulose sodium salt as sticker @ 0.5 per cent. Then the concentration of the bacteria was adjusted to $10^8$cfu/ml and used as inoculum.

### 3.14.3. Seed treatment

Rice seeds were surface sterilized with one per cent sodium hypochlorite solution for one minute, washed with three changes of sterile distilled water. Then seeds were treated with promising bacterial antagonists/bacterial bio control agent/ bactericides separately and sown in nursery beds.

### 3.14.4. Seedling root dip and transplanting

Rice seedlings (20 days old) were uprooted, removed the soil particles and dipped in bacterial suspension/biocontrol agent having required concentration. Seedlings @ two/hill were transplanted to the plots of 3 m x 2 m with a spacing of 15 cm x10 cm. Each treatment was replicated thrice. Plots without any treatment served as the absolute control.

### 3.14.5. Preparation of pathogen inoculum

The virulent isolate of *Xoo* from Polpully of Palakkad district was grown on PSA medium. Culture of 48 h old was used for the inoculation of the rice plants at the time of active tillering stage. The concentration of the bacterial cells was adjusted to $10^8$ $ml^{-1}$.

### 3.14.6. Artificial inoculation of the pathogen *Xoo*

Culture of the pathogen (48 h old) was used for artificial inoculation on rice plants. All the treatments including absolute control were inoculated with the pathogen at 27 DAT by clip inoculation method.

### 3.14.7. Foliar spray

Foliar spray with selected bacterial antagonists, bacterial bio control agent, antibiotics, chemicals and organics were given on 30 DAT of the pathogen and repeated at 45 DAT.

## 3.15. Disease reaction due to various treatments

The observations on PDI and PDS were recorded from five plants taken from four different corners and at the centre of the plot and CI was calculated as mentioned in 3.1. at 60 DAT.

## 3.16. Effect of different treatments on biometric characters

### 3.16.1. Growth characters

#### 3.16.1.1. Plant height

At 60 DAT, the height of five plants in each treatment was measured from the base of the plant to the tip of the top leaf. The mean height was computed and expressed in cm.

**3.16.1.2. Number of tillers**

The number of productive tillers was counted from five hills in each treatment at 60 DAT. The mean number of tillers was computed and expressed as number $hill^{-1}$.

**3.16.1.3. Root characters**

**3.16.1.4. Root length**

Lengths of roots in five randomly selected and uprooted hills at the time of harvest, were measured and the mean expressed in cm.

**3.16.1.5. Root dry weight**

The roots from five randomly selected and uprooted hills were washed free of soil and separated from the stem. They were first air dried and then oven dried at 60°C to constant weight. The root dry weight was recorded and the mean expressed in g $hill^{-1}$.

**3.16.2. Yield attributes**

**3.16.2.1. Panicle length**

Length of five randomly selected panicles was measured and the mean expressed in cm.

**3.16.2.2. Number of filled grains/panicle**

The number of filled grains of five randomly selected panicles was taken and the mean was calculated and expressed as number of filled grains/panicle.

**3.16.2.3. Thousand grain weight**

One thousand grains were collected, at random, from the produce of each plot and their weight was recorded in g.

**3.16.2.4. Grain yield**

The grains from each plot, after winnowing and cleaning, were weighed and grain yield was computed at 13 per cent moisture and expressed in kg/ha.

**3.16.2.5. Straw yield**

The straw from each plot was sun dried uniformly, weighed and expressed in kg/ha.

**3.17. Statistical analysis**

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986) and SPSS package.

# Results

# 4. RESULTS

## 4.1. Survey on the occurrence of bacterial blight disease in three districts of Kerala

A survey was conducted in three districts of Kerala *viz.*, Alappuzha, Palakkad and Thrissur during September 2007 to study the occurrence of bacterial blight disease and the results are presented in Table 1. Per cent Disease Incidence (PDI), Per cent Disease Severity (PDS) and Co-efficient of Infection (CI) values in 14 rice growing locations ranged from 20.94 – 60.34, 57.05 – 92.42 and 11.97 – 55.77 respectively. The maximum PDI value was recorded in paddy fields of Erattakulam (60.34) followed by Athimani (53.95) and Parali (53.87) fields and were on par with each other. This was followed by Polpully area with PDI value of 49.77. Nenmara and Edathua were ranked next and were on par as evidenced by the PDI values of 47.55 and 46.16 respectively. PDS was also found to be maximum in paddy fields of Erattakulam (92.42) followed by Nenmara (88.63), Athimani (87.65) and Parali (87.18) areas and were found on par with each other. Pattambi (85.72), Polpully (85.49) and Edathua (85.18) ranked next as evidenced by the PDS values. Based on PDI and PDS, Co-efficient of infection (CI) was calculated for the 14 locations. The maximum CI value of 55.77 was recorded from Erattakulam area which was followed by Athimani (47.30) and Parali (46.96). Polpully (42.55) and Nenmara (42.13) areas ranked next followed by Edathua (38.99) and Kodakara (37.40). Pattambi (36.80) and Mannuthy (35.72) were on par with each other followed by Akamala (34.61) and Karuvatta (33.77). Manchira recorded the CI value of 27.26. Paddy fields of Kodallur and Moncompu recorded least CI values of 11.97 and 12.29 respectively.

In conclusion, out of the 14 rice growing locations surveyed on the occurrence of bacterial blight in two popular varieties *viz.*, Uma (MO-16) and Jyothi (PTB-39), the following five locations *viz.*, Erattakulam, Athimani, Parali, Polpully and Nenmara in Palakkad district recorded susceptible reaction with CI values ranged from 42.13-55.77(Plate 1).The rice fields of seven locations *viz.*, Edathua, Kodakara, Mannuthy, Pattambi, Karuvatta, Akamala and Manchira recorded moderately susceptible reaction with CI values ranged from 27.26 - 38.99. Kodallur and Moncombu fields recorded moderately resistant reaction to bacterial blight with lesser CI values of 12.29 and 11.97 respectively.

**Table1. Occurrence of bacterial blight of rice in three districts of Kerala**

| Sl.No. | Place | Variety | Stage of the crop | PDI | PDS | CI value | Disease reaction |
|---|---|---|---|---|---|---|---|
| **PALAKKAD DISTRICT** | | | | | | | |
| 1 | Athimani | Uma | Flowering stage | $53.95^{b}$ | $87.65^{b}$ | $47.30^{b}$ | Susceptible |
| 2 | Erattakulam | Uma | Flowering stage | $60.34^{a}$ | $92.42^{a}$ | $55.77^{a}$ | Susceptible |
| 3 | Kodallur | Uma | Flowering stage | $21.18^{i}$ | $57.95^{g}$ | $12.29^{h}$ | Moderately Resistant |
| 4 | Manchira | Jyothi | Flowering stage | $34.17^{h}$ | $73.31^{f}$ | $27.26^{g}$ | Moderately Susceptible |
| 5 | Nenmara | Jyothi | Flowering stage | $47.55^{d}$ | $88.63^{b}$ | $42.13^{c}$ | Susceptible |
| 6 | Parali | Jyothi | Flowering stage | $53.87^{b}$ | $87.18^{b}$ | $46.96^{b}$ | Susceptible |
| 7 | Pattambi | Jyothi | Flowering stage | $42.95^{f}$ | $85.72^{c}$ | $36.80^{e}$ | Moderately Susceptible |
| 8 | Polpully | Uma | Flowering stage | $49.77^{c}$ | $85.49^{c}$ | $42.55^{c}$ | Susceptible |
| **THRISSUR DISTRICT** | | | | | | | |
| 9 | Akamala | Jyothi | Flowering stage | $41.55^{g}$ | $83.21^{d}$ | $34.61^{f}$ | Moderately Susceptible |
| 10 | Kodakara | Jyoyhi | Flowering stage | $44.83^{e}$ | $83.43^{d}$ | $37.40^{d}$ | Moderately Susceptible |
| 11 | Mannuthy | Uma | Flowering stage | $43.62^{f}$ | $81.93^{e}$ | $35.72^{e}$ | Moderately Susceptible |
| **ALAPPUZHA DISTRICT** | | | | | | | |
| 12 | Edathua | Uma | Flowering stage | $46.16^{d}$ | $85.18^{c}$ | $38.99^{d}$ | Moderately Susceptible |
| 13 | Karuvatta | Uma | Flowering stage | $41.25^{g}$ | $81.89^{e}$ | $33.77^{f}$ | Moderately Susceptible |
| 14 | Moncombu | Uma | Flowering stage | $20.94^{i}$ | $57.05^{g}$ | $11.97^{h}$ | Moderately Resistant |

Values under same subscript form a homogenous sub group

**Plate 1. Symptoms of bacterial blight in different locations**

**Athimani**

**Erattakulam**

**Nenmara**

**Parali**

**Polpully**

**Mannuthy**

## 4.2. Symptomatology

### 4.2.1. Symptomatology under natural condition

Two phases of symptoms *viz*., leaf blight and kresek developed under natural condition were recorded during the survey conducted for the collection of diseased specimens from 14 locations of three districts of Kerala.

The leaf blight symptoms first appeared as tiny water soaked lesions or stripes started from the leaf margins near its tip. As the disease advanced, the water soaked lesions turned yellow, enlarged progressively and extended to one or both sides of the leaf margins (Plate 2a). Long wavy stripes covering the entire leaf margins leaving a small greenish portion in the midrib of the leaves, the characteristic symptom of the bacterial blight was noticed in all the locations surveyed. Occasionally, the yellow lesions extended along the midrib leaving greenish leaf margins were also observed. Later the yellowish lesions changed to white or straw in colour. The infection on the ear head, caused grey to light brown lesions on glumes, resulting in infertility and low grain quality.

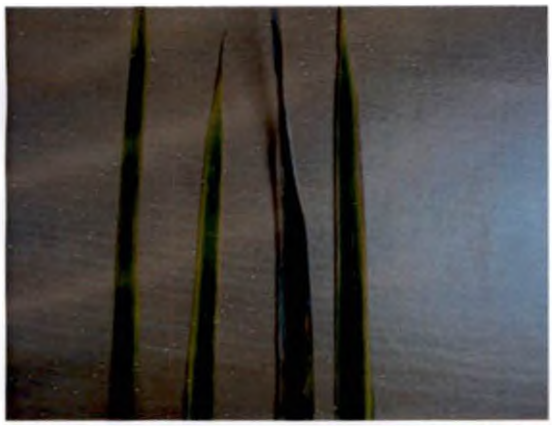
The kresek symptoms were observed in the nursery as greyish green lesions on lower leaves. As the disease advanced, the infected leaves rolled up along the midrib, withered and wilted resulting in the death of the plants.

### 4.2.2. Symptomatology under artificial conditions

To study the leaf blight symptom, 45 days old Jyothi (PTB-39) plants were inoculated separately with 14 isolates of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) obtained from different locations. All the isolates showed initial symptom of yellowing at the cut ends at three to five days interval. Later, the yellowish lesions extended on the both margins of the leaf and appeared as a characteristic wavy lesions leading to whitening and drying of the leaves (Plate 2b).

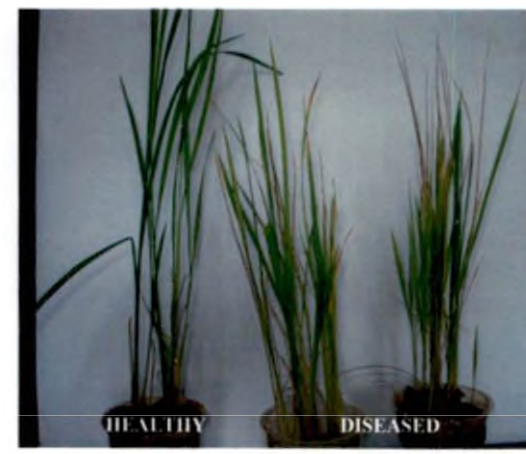
The kresek symptom was studied on 21 days old seedlings of the variety Jyothi (PTB-39) grown in plastic pots. The initial symptom of pale yellow leaves followed by rolling along

**Plate 2. Symptomatology of bacterial blight disease**



**a. Leaf blight symptom under natural condition**

**b. Leaf blight symptom on artificial inoculation**




**c. Kresek symptom on artificial inoculation**

the midrib was appeared from fifth DAI to fifteenth DAI. Finally wilting and drying of the seedlings occurred (Plate 2c).

### 4.3. Collection, isolation, naming of different isolates of *Xoo* and testing their pathogenicity

Single colonies of *Xoo* were picked up from the PSA medium and were purified by streaking on the same medium. Each single colony was maintained as a single isolate and was stored in duplicates in sterile distilled water at 4°C. The details of the isolates obtained are presented in Table 2. A total of 52 isolates of *Xoo* were obtained from infected leaf samples collected from three districts of Kerala. Among these, 14 isolates representing 14 locations were selected for further studies.

The pathogenicity of the 14 isolates was proved by inoculating them on healthy rice seedlings of a highly susceptible variety Jyothi (PTB-39) at maximum tillering stage. Typical symptoms of leaf blight were observed within two to five days of inoculation.The pathogens were reisolated from these plants and compared with the original isolates.

### 4.4. Cultural, morphological and biochemical characteristics of rice bacterial blight isolates

The cultural and morphological characters of the pathogen were studied on PSA medium and the results are presented in Table 3 (Plate 3). The colony size of the 14 isolates varied from 1-3 mm diameter. Eight isolates *viz*., Athimani (XAMI-1), Erattakulam (XERM - 5), Kodallur (XKOR-3), Manchira (XMRA-4), Parali (XPAI-5), Akamala (XAKA-4), Mannuthy (XMTY-2) and Karuvatta (XKVA-2) had colonies of 1 to 2 mm in diameter, whereas the remaining six isolates *viz*., Nenmara (XNRA-5), Pattambi (XPTB-2), Polpully (XPLY-3), Kodakkara (XKDA-3), Edathua (XEDA-3) and Moncombu (XMOU-2) had colonies of 1 to 3 mm in diameter. Shape of the colony was circular, convex with round margin on 12 isolates, where as the colonies of Manchira (XMRA-2) and Moncombu (XMOU-1) isolates were circular, convex with irregular margin. Pigmentation of the colonies of 12 isolates

**Table 2.** ***X. oryzae*** **pv.** ***oryzae*** **(*Xoo*) isolates obtained from different locations of Kerala**

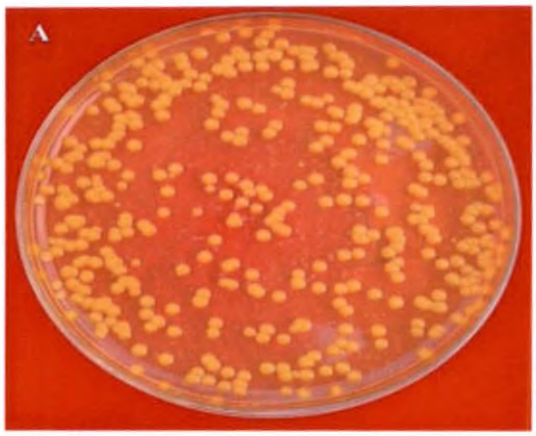
| Sl. No. | Isolate number | Place of collection | Variety | No. of isolates obtained |
|---|---|---|---|---|
| **PALAKKAD DISTRICT** | | | | |
| 1 | XAMI (1-5) | Athimani | Uma | 5 |
| 2 | XERM (1-5) | Erattakulam | Uma | 5 |
| 2 | XKOR (1-3) | Kodallur | Uma | 3 |
| 4 | XMRA (1-4) | Manchira | Jyothi | 4 |
| 5 | XNRA (1-5) | Nenmara | Jyothi | 5 |
| 6 | XPAI (1-5) | Parali | Jyothi | 5 |
| 7 | XPTB (1-4) | Pattambi | Jyothi | 4 |
| 8 | XPLY (1-5) | Polpully | Uma | 5 |
| **THRISSUR DISTRICT** | | | | |
| 9 | XAKA (1-4) | Akamala | Uma | 4 |
| 10 | XKDA (1-3) | Kodakara | Uma | 3 |
| 11 | XMTY (1-2) | Mannuthy | Uma | 2 |
| **ALAPPUZHA DISTRICT** | | | | |
| 12 | XEDA (1-3) | Edathua | Jyothi | 3 |
| 13 | XKVA (1-2) | Karuvatta | Jyothi | 2 |
| 14 | XMOU (1-2) | Moncombu | Uma | 2 |

**Table 3. Cultural and morphological characteristics of rice bacterial blight (*Xoo*) isolates**

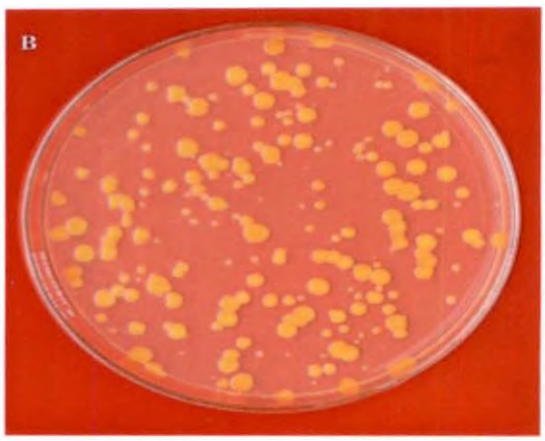
| Sl. No. | Isolate number | Colony size (diam.) | Colony shape | Pigmentation | *Sliminess | Incubation period (h) |
|---|---|---|---|---|---|---|
| **PALAKKAD ISOLATES** | | | | | | |
| 1 | XAMI-3 | 1 to 2mm | Circular,convex with round margin | Dark yellow | +++ | 36 |
| 2 | XERM-1 | 1 to 2mm | Circular,convex with round margin | Dark yellow | +++ | 48 |
| 3 | XKOR-3 | 1 to 2 mm | Circular,convex with round margin | Light yellow | +++ | 24 |
| 4 | XMRA-2 | 1 to 2 mm | Circular, convex with irregular margin | Dark yellow | +++ | 24 |
| 5 | XNRA-1 | 1 to 3 mm | Circular,convex with round margin | Dark yellow | +++ | 48 |
| 6 | XPAI-3 | 1 to 2 mm | Circular,convex with round margin | Dark Yellow | +++ | 48 |
| 7 | XPTB- 4 | 1 to 3 mm | Circular,convex with round margin | Dark yellow | +++ | 24 |
| 8 | XPLY-5 | 1 to 3 mm | Circular,convex with round margin | Dark Yellow | +++ | 36 |
| **THRISSUR ISOLATES** | | | | | | |
| 9 | XAKA-2 | 1 to 2 mm | Circular,convex with round margin | Light yellow | ++ | 24 |
| 10 | XKDA-1 | 1 to 3 mm | Circular,convex with round margin | Dark yellow | +++ | 24 |
| 11 | XMTY-2 | 1 to 2 mm | Circular,convex with round margin | Dark yellow | +++ | 24 |
| **ALAPPUZHA ISOLATES** | | | | | | |
| 12 | XEDA-3 | 1 to 3 mm | Circular,convex with round margin | Light yellow | ++ | 24 |
| 13 | XKVA-1 | 1 to 2 mm | Circular,convex with round margin | Dark yellow | +++ | 24 |
| 14 | XMOU-1 | 1 to 3 mm | Circular, convex with irregular margin | Dark yellow | +++ | 24 |

* +++ Good; ++ Moderate

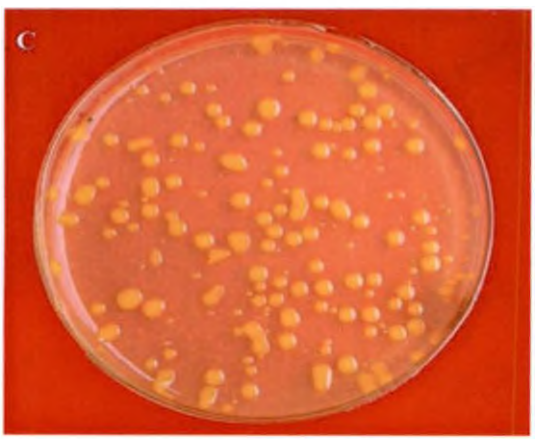
**Plate 3. Different isolates of *Xanthomonas oryzae* pv. *oryzae***




**a. Athimani(XAMI-3)**

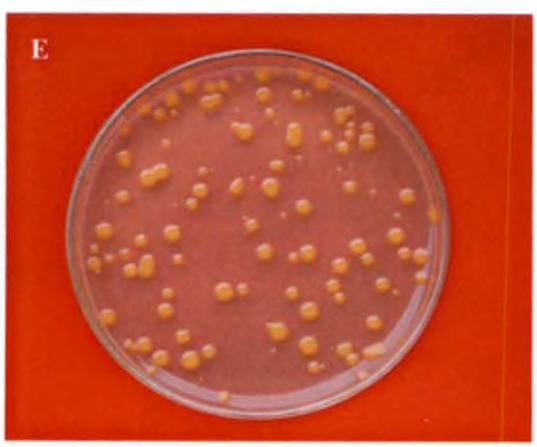



**b. Erattakulam(XERM-1)**

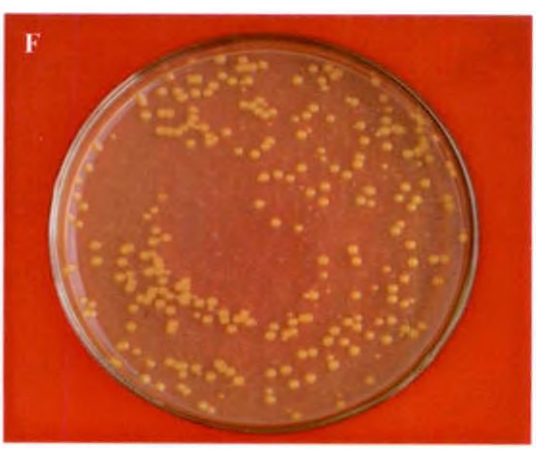



**c. Nenmara(XNRA-1)**


**d. Parali(XPLY-5)**




**e. Polpully(XPLY-5)**




**f. Mannuthy(XMTY-2)**

were dark yellow with good sliminess, where as the Akamala (XAKA-2) and Edathua (XEDA-3) isolates were light yellow in colour with moderate sliminess.

Incubation period of the 14 isolates varied from 24 h to 48 h. Colonies of nine isolates *viz*., Kodallur (XKOR-3), Manchira (XMRA-2) and Pattambi (XPTB-4) from Palakkad district, Akamala (XAKA-2), Kodakara (XKDA-1) and Mannuthy (XMTY-2) from Thrissur district, Edathua (XEDA-3), Karuvatta (XKVA-1) and Moncombu (XMOU-1) of Alappuzha district were developed at 24 h of incubation. The two isolates *viz*., Athimani (XAMI-3) and Polpully (XPLY-5) had formed colonies at 36 h of incubation, where as the three isolates *viz*., Erattakulam (XERM-1), Nenmara (XNRA-1) and Polpully (XPLY-5) from Palakkad district took 48 h of incubation for colony formation.

### 4.4.1. Biochemical characters of the bacterial blight isolates

Biochemical studies revealed that all the 14 isolates reacted positively to starch hydrolysis, levan formation and arginine dihydrolase tests and produced pink colour rods on Gram staining and were found Gram negative. The 14 isolates showed positive reaction to KOH test by forming thick thread which confirmed the Gram negative reaction of these isolates.

The isolates differed in citrate utilization, gelatin liquefaction and $H_2S$ production (Table 4). Parali (XPAI-3) and Pattambi (XPTB-4) isolates could utilize the citrate. Except Kodallur (XKOR-3) and Nenmara (XNRA-1) isolates, all the other twelve isolates could liquefy gelatin. $H_2S$ was produced by Parali (XPAI-3) isolate alone. The isolates differed slightly in carbohydrates utilization. All the isolates showed positive reaction to lactose and sucrose utilization. Polpully (XPLY-5) and Edathua (XEDA-3) isolates showed positive reaction to xylose where as Polpully isolate (XPLY-5) alone could utilize maltose. Kodakara isolate (XKDA-1) alone could utilize fructose. Manchira (XMRA-2) and Polpully (XPLY-5) isolates showed positive reaction to dextrose, galactose, raffinose, trehalose and melibiose utilization. All the isolates could utilize sucrose where as Kodallur (XKOR-3), Manchira

**Table 4. Biochemical characters of the bacterial blight isolates**

| Biochemical tests/ Isolates | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram staining | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Starch hydrolysis | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Levan formation | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Citrate utilization | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| Gelatin liquefaction | + | + | - | + | - | + | + | + | + | + | + | + | + | + |
| Arginine dihydrolase | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| $H_2S$ production | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| VP test | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| MR test | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3% KOH solubility test | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nitrate utilization | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease production | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Oxidase test | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Xylose | - | - | - | - | - | - | - | + | - | - | - | + | - | - |
| Maltose | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| Fructose | - | - | - | - | - | - | - | - | - | + | - | - | - | - |
| Dextrose | - | - | - | + | - | - | - | + | - | - | - | - | - | - |
| Galactose | - | - | - | + | - | - | - | + | - | - | - | - | - | - |
| Raffinose | - | - | - | + | - | - | - | + | - | - | - | - | - | - |
| Trehalose | - | - | - | + | - | - | - | + | - | - | - | - | - | - |
| Melibiose | - | - | - | + | - | - | - | + | - | - | - | - | - | - |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Arabinose | - | - | + | + | - | - | - | + | - | - | - | - | - | - |
| Mannose | - | - | - | - | - | - | - | + | - | - | - | - | - | - |

1. XAMI-3 2. XERM-1 3. XKOR-3 4. XMRA-2 5. XNRA-1 6. XPAI-3
7. XPTB-4 8. XPLY-5 9. XAKA-2 10. XKDA-1 11. XMTY-2 12. XEDA-3
13. XKVA-1 14. XMOU-1

(XMRA-2) and Polpully (XPLY-5) could utilize L-arabinose. Polpully isolate (XPLY-5) alone could utilize mannose.

From the cultural, morphological and biochemical studies, the pathogen causing bacterial blight disease is identified as *Xanthomonas oryzae* pv. *oryzae* and these studies revealed that there were variations among the isolates of *Xoo* obtained from different locations.

### 4.5. Studies on the pathogenic variability of *Xoo* isolates

#### 4.5.1. Studies on leaf blight reaction of *Xoo* isolates on twenty rice varieties of Kerala

Twenty rice varieties as depicted in 3.5.1 were artificially inoculated with the 14 isolates of *Xoo* obtained from respective districts (Alappuzha, Palakkad and Thrissur) and the pathogenic variability was studied during September 2007 (Plate 4).

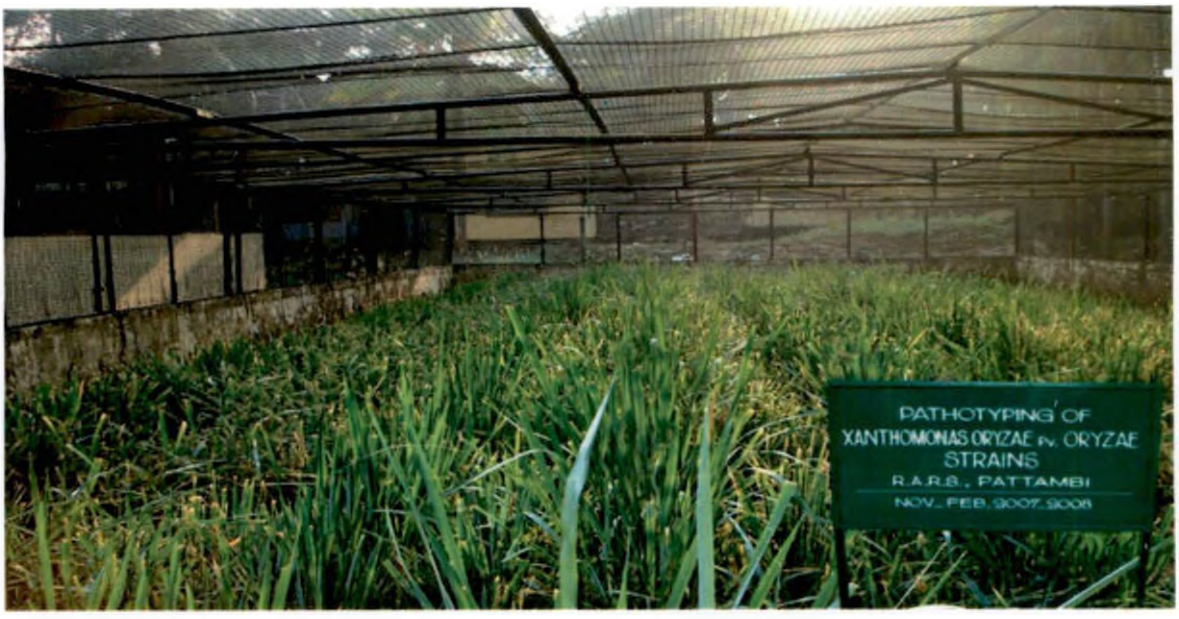
**4.5.1.1. Athimani isolate** (XAMI-3)

The results are presented in Table 5.1. From the data, it was found that none of the varieties were completely free from infection by the isolate. Nineteen varieties showed CI values ranging from 39.49 to 49.97 and found to be susceptible to the isolate and showed the lesion length ranging from 15.02 cm to 20.36 cm. The variety Makaram showed a CI value of 2.16 and was found to be resistant to the isolate and showed the lesion length of 1.92 cm only. All the 19 varieties showed the initial symptom of yellow lesion in two days, whereas the variety Makaram took 15 days to produce the symptom.

**4.5.1.2. Erattakulam isolate** (XERM-1)

The results presented in Table 5.2, revealed that 19 varieties showed the CI values ranging from 42.84 to 57.49 and found susceptible to the isolate and showed the lesion length ranging from 18.40 to 22.16 cm. The variety Makaram showed the CI value of 1.25 and found to be resistant to the isolate and showed the lesion length of 1.16 cm. All the 19 varieties

**Plate 4. Pathogenic variability studies of *Xoo* isolates**




**a. Net house view of the experiment**

**b. Initial symptom expression of Athimani isolate (XAMI-3)**


**c. Advanced stages of symptom expression of Athimani isolate (XAMI-3)**

**Table 5.1. Bacterial blight disease reaction of Athimani isolate (XAMI-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 2 | Yellow lesion | $53.80^{e}$ | $91.03^{e}$ | $48.97^{h}$ | $19.52^{i}$ | Yellowing, whitening and drying |
| 2 | Anashwara | 2 | Yellow lesion | $53.18^{e}$ | $90.23^{b}$ | $47.98^{h}$ | $18.80^{g}$ | Yellowing, whitening and drying |
| 3 | Annapoorna | 2 | Yellow lesion | $51.87^{e}$ | $87.45^{b}$ | $45.36^{f}$ | $17.18^{f}$ | Yellowing, whitening and drying |
| 4 | Aswathi | 2 | Yellow lesion | $53.32^{e}$ | $88.69^{b}$ | $47.28^{h}$ | $18.82^{g}$ | Yellowing, whitening and drying |
| 5 | Bhadra | 2 | Yellow lesion | $54.62^{e}$ | $91.49^{d}$ | $49.97^{i}$ | $20.36^{j}$ | Yellowing, whitening and drying |
| 6 | Harsha | 2 | Yellow lesion | $52.92^{e}$ | $90.42^{b}$ | $47.85^{h}$ | $18.94^{h}$ | Yellowing, whitening and drying |
| 7 | Jaya | 2 | Yellow lesion | $52.59^{e}$ | $89.59^{b}$ | $47.11^{h}$ | $18.32^{g}$ | Yellowing, whitening and drying |
| 8 | Jyothi | 2 | Yellow lesion | $51.95^{e}$ | $88.80^{b}$ | $46.13^{g}$ | $16.54^{e}$ | Yellowing, whitening and drying |
| 9 | Kairali | 2 | Yellow lesion | $53.21^{e}$ | $89.27^{b}$ | $47.50^{h}$ | $18.26^{g}$ | Yellowing, whitening and drying |
| 10 | Kanchana | 2 | Yellow lesion | $51.72^{e}$ | $87.79^{b}$ | $45.40^{f}$ | $17.10^{f}$ | Yellowing, whitening and drying |
| 11 | Karuna | 2 | Yellow lesion | $48.66^{d}$ | $87.71^{b}$ | $42.67^{d}$ | $16.08^{d}$ | Yellowing, whitening and drying |
| 12 | Kunju Kunju Varna | 2 | Yellow lesion | $45.88^{c}$ | $87.43^{b}$ | $40.11^{b}$ | $15.06^{b}$ | Yellowing, whitening and drying |
| 13 | Makaram | 15 | Yellow lesion | $6.23^{a}$ | $34.77^{a}$ | $2.16^{a}$ | $1.92^{a}$ | Yellowing, whitening and drying |
| 14 | Manupriya | 2 | Yellow lesion | $45.96^{c}$ | $91.34^{e}$ | $41.97^{c}$ | $15.22^{b}$ | Yellowing, whitening and drying |
| 15 | Matta Triveni | 2 | Yellow lesion | $48.66^{d}$ | $87.71^{b}$ | $42.67^{d}$ | $15.06^{b}$ | Yellowing, whitening and drying |
| 16 | Neeraja | 2 | Yellow lesion | $45.88^{c}$ | $87.43^{b}$ | $40.11^{b}$ | $15.22^{b}$ | Yellowing, whitening and drying |
| 17 | Rohini | 2 | Yellow lesion | $44.10^{b}$ | $89.53^{b}$ | $39.48^{b}$ | $15.02^{b}$ | Yellowing, whitening and drying |
| 18 | Sabari | 2 | Yellow lesion | $45.99^{b}$ | $88.39^{b}$ | $40.65^{b}$ | $16.08^{d}$ | Yellowing, whitening and drying |
| 19 | Swetha | 2 | Yellow lesion | $48.00^{d}$ | $90.18^{b}$ | $43.28^{e}$ | $15.78^{c}$ | Yellowing, whitening and drying |
| 20 | Uma | 2 | Yellow lesion | $51.97^{e}$ | $91.77^{e}$ | $47.69^{h}$ | $18.74^{g}$ | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.2. Bacterial blight disease reaction of Erattakulam isolate (XERM-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 2 | Yellow lesion | $57.94^{i}$ | $92.66^{j}$ | $53.68^{h}$ | $21.06^{e}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 2 | Yellow lesion | $55.76^{g}$ | $91.37^{h}$ | $50.94^{i}$ | $20.42^{c}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 2 | Yellow lesion | $54.61^{e}$ | $89.51^{e}$ | $48.88^{e}$ | $20.50^{d}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 2 | Yellow lesion | $54.01^{d}$ | $90.07^{f}$ | $48.64^{e}$ | $21.90^{f}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 2 | Yellow lesion | $55.38^{f}$ | $90.30^{g}$ | $50.00^{f}$ | $20.70^{c}$ | Yellowing,whitening and drying |
| 6 | Harsha | 2 | Yellow lesion | $54.54^{e}$ | $88.59^{d}$ | $48.31^{d}$ | $19.54^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 2 | Yellow lesion | $51.19^{b}$ | $85.87^{b}$ | $43.95^{b}$ | $20.18^{c}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 2 | Yellow lesion | $51.40^{b}$ | $86.02^{b}$ | $44.21^{b}$ | $19.36^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 2 | Yellow lesion | $51.05^{b}$ | $85.29^{b}$ | $43.54^{b}$ | $18.48^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 2 | Yellow lesion | $52.29^{b}$ | $84.77^{b}$ | $44.32^{b}$ | $18.86^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 2 | Yellow lesion | $51.88^{b}$ | $86.74^{b}$ | $45.00^{b}$ | $20.72^{c}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 2 | Yellow lesion | $50.56^{b}$ | $84.74^{b}$ | $42.84^{b}$ | $21.92^{f}$ | Yellowing,whitening and drying |
| 13 | Makaram | 15 | Yellow lesion | $4.76^{a}$ | $26.19^{a}$ | $1.04^{a}$ | $1.16^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 2 | Yellow lesion | $53.61^{e}$ | $86.85^{b}$ | $46.56^{c}$ | $21.68^{f}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 2 | Yellow lesion | $51.83^{b}$ | $87.39^{c}$ | $45.29^{b}$ | $21.84^{f}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 2 | Yellow lesion | $53.10^{b}$ | $87.78^{c}$ | $46.61^{c}$ | $21.56^{f}$ | Yellowing,whitening and drying |
| 17 | Rohini | 2 | Yellow lesion | $52.67^{b}$ | $87.67^{c}$ | $46.17^{c}$ | $20.70^{c}$ | Yellowing,whitening and drying |
| 18 | Sabari | 2 | Yellow lesion | $52.24^{b}$ | $86.78^{b}$ | $45.33^{b}$ | $18.40^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 2 | Yellow lesion | $56.64^{h}$ | $91.69^{i}$ | $51.93^{g}$ | $21.92^{f}$ | Yellowing,whitening and drying |
| 20 | Uma | 2 | Yellow lesion | $60.48^{j}$ | $95.04^{k}$ | $57.48^{i}$ | $22.16^{g}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

showed the initial symptom of yellow lesion in two days, whereas the variety Makaram took 15 days to produce the symptom.

**4.5.1.3. Kodallur isolate** (XKOR-3)

As seen in Table 5.3, all the nineteen varieties showed the CI value ranging from 9.33 to 11.81 and were found to be moderately resistant to the isolate and showed the lesion length ranging from 5.22 to 6.50 cm. Makaram showed the CI value of 0.21 and was found resistant to the isolate and showed the lesion length of 0.4 cm. All the 19 varieties showed the symptom in five days, whereas the variety Makaram took 20 days to produce the symptom

**4.5.1.4. Manchira isolate** (XMRA-2)

As per the results presented in Table 5.4, it was found that none of varieties were found completely free from infection. All the 19 varieties showed the CI values ranging from 27.84 to 37.03 and were found moderately susceptible to the bacterial blight isolate. All the 19 varieties showed lesion length ranging from 10.88 cm to 13.66 cm. Makaram showed the CI value of 0.81 and was found to be resistant to the isolate and the lesion length was 0.64 cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took 16 days to produce the symptom.

**4.5.1.5. Nenmara isolate** (XNRA-1)

As seen in Table 5.5, all the 19 varieties showed the CI values ranging from 40.43 to 46.37 and were susceptible to the isolate, showing lesion length ranging from 15.06 to 18.18 cm. Makaram showed the CI value of 1.28 and found to be highly resistant to the isolate. The lesion length was 1.32 cm. All the 19 varieties showed the initial symptom of yellow lesion in two days, whereas the variety Makaram took 15 days to produce the symptom.

**Table 5.3. Bacterial blight disease reaction of Kodallur isolate (XKOR-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 5 | Yellow lesion | $18.16^{e}$ | $51.40^{b}$ | $9.33^{b}$ | $5.66^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 5 | Yellow lesion | $20.12^{e}$ | $52.90^{b}$ | $10.64^{d}$ | $6.04^{d}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 5 | Yellow lesion | $19.37^{d}$ | $53.27^{b}$ | $10.31^{c}$ | $5.86^{d}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 5 | Yellow lesion | $17.88^{e}$ | $53.63^{b}$ | $9.58^{b}$ | $5.64^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 5 | Yellow lesion | $19.02^{d}$ | $53.33^{b}$ | $10.14^{c}$ | $5.94^{d}$ | Yellowing,whitening and drying |
| 6 | Harsha | 5 | Yellow lesion | $19.23^{d}$ | $53.42^{b}$ | $10.23^{c}$ | $5.94^{d}$ | Yellowing,whitening and drying |
| 7 | Jaya | 5 | Yellow lesion | $18.16^{e}$ | $52.51^{b}$ | $9.53^{b}$ | $5.76^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 5 | Yellow lesion | $17.36^{b}$ | $54.26^{c}$ | $9.41^{b}$ | $5.72^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 5 | Yellow lesion | $18.06^{e}$ | $52.88^{b}$ | $9.55^{b}$ | $5.90^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 5 | Yellow lesion | $17.79^{e}$ | $53.71^{b}$ | $9.55^{b}$ | $5.60^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 5 | Yellow lesion | $20.77^{e}$ | $56.89^{d}$ | $11.81^{e}$ | $6.44^{e}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 5 | Yellow lesion | $18.34^{e}$ | $53.44^{b}$ | $9.80^{b}$ | $5.70^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 20 | Yellow lesion | $0.15^{a}$ | $1.43^{a}$ | $0.21^{a}$ | $0.40^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 5 | Yellow lesion | $18.38^{e}$ | $52.74^{b}$ | $9.69^{b}$ | $5.82^{c}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 5 | Yellow lesion | $18.53^{e}$ | $53.85^{b}$ | $9.97^{b}$ | $5.88^{d}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 5 | Yellow lesion | $18.06^{e}$ | $52.88^{b}$ | $9.55^{b}$ | $5.64^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 5 | Yellow lesion | $17.60^{b}$ | $55.34^{c}$ | $9.73^{b}$ | $5.90^{d}$ | Yellowing,whitening and drying |
| 18 | Sabari | 5 | Yellow lesion | $20.12^{e}$ | $52.90^{b}$ | $10.64^{d}$ | $5.86^{d}$ | Yellowing,whitening and drying |
| 19 | Swetha | 5 | Yellow lesion | $19.23^{d}$ | $53.42^{b}$ | $10.23^{c}$ | $5.22^{b}$ | Yellowing,whitening and drying |
| 20 | Uma | 5 | Yellow lesion | $20.28^{e}$ | $56.81^{d}$ | $11.52^{e}$ | $6.50^{e}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.4. Bacterial blight disease reaction of Manchira isolate (XMRA-2) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $41.86^{g}$ | $84.21^{b}$ | $35.25^{e}$ | $13.16^{e}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $43.92^{i}$ | $84.25^{b}$ | $37.00^{h}$ | $13.58^{f}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $42.64^{h}$ | $85.96^{c}$ | $36.65^{g}$ | $13.66^{g}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $42.03^{g}$ | $85.91^{c}$ | $36.10^{f}$ | $13.40^{f}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $41.73^{g}$ | $86.06^{d}$ | $35.91^{f}$ | $13.30^{f}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $39.19^{e}$ | $83.68^{b}$ | $32.79^{e}$ | $12.42^{e}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $37.82^{b}$ | $84.22^{b}$ | $31.85^{b}$ | $12.18^{h}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $34.79^{b}$ | $81.25^{b}$ | $28.26^{b}$ | $10.92^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $38.04^{b}$ | $83.35^{b}$ | $31.70^{b}$ | $12.16^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $34.03^{b}$ | $81.72^{b}$ | $27.80^{b}$ | $10.88^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $36.25^{b}$ | $83.78^{b}$ | $30.37^{b}$ | $11.72^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $36.86^{b}$ | $82.61^{b}$ | $30.45^{b}$ | $11.76^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 16 | Yellow lesion | $3.66^{a}$ | $22.25^{a}$ | $0.81^{a}$ | $0.64^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $38.83^{c}$ | $82.34^{b}$ | $31.97^{b}$ | $12.12^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $38.75^{c}$ | $83.77^{b}$ | $32.46^{b}$ | $12.78^{d}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $34.96^{b}$ | $81.88^{b}$ | $28.62^{b}$ | $11.28^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $39.45^{d}$ | $82.76^{b}$ | $32.64^{e}$ | $12.52^{e}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $36.23^{b}$ | $81.97^{b}$ | $29.69^{b}$ | $11.58^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $40.31^{e}$ | $83.56^{b}$ | $33.68^{d}$ | $12.76^{d}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $41.01^{f}$ | $83.72^{b}$ | $34.33^{d}$ | $12.94^{d}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.5. Bacterial blight disease reaction of Nenmara isolate (XNRA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 2 | Yellow lesion | $52.84^{b}$ | $88.14^{c}$ | $46.57^{f}$ | $18.18^{g}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 2 | Yellow lesion | $51.37^{b}$ | $86.98^{c}$ | $44.68^{e}$ | $16.92^{e}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 2 | Yellow lesion | $51.26^{b}$ | $87.10^{c}$ | $44.64^{e}$ | $17.26^{f}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 2 | Yellow lesion | $51.90^{b}$ | $86.10^{c}$ | $44.68^{e}$ | $16.84^{d}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 2 | Yellow lesion | $49.23^{b}$ | $87.33^{c}$ | $42.99^{d}$ | $16.38^{d}$ | Yellowing,whitening and drying |
| 6 | Harsha | 2 | Yellow lesion | $48.29^{b}$ | $84.93^{c}$ | $41.01^{b}$ | $15.06^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 2 | Yellow lesion | $49.24^{b}$ | $85.53^{c}$ | $42.11^{c}$ | $15.58^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 2 | Yellow lesion | $48.97^{b}$ | $85.31^{c}$ | $41.77^{c}$ | $15.70^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 2 | Yellow lesion | $49.54^{b}$ | $85.72^{c}$ | $42.46^{c}$ | $16.20^{c}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 2 | Yellow lesion | $50.32^{b}$ | $86.60^{c}$ | $43.57^{d}$ | $16.14^{c}$ | Yellowing,whitening and drying |
| 11 | Karuna | 2 | Yellow lesion | $48.75^{b}$ | $87.52^{c}$ | $42.66^{c}$ | $16.34^{d}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 2 | Yellow lesion | $50.04^{b}$ | $87.03^{c}$ | $43.54^{d}$ | $15.34^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 15 | Yellow lesion | $4.53^{a}$ | $28.32^{a}$ | $1.28^{a}$ | $1.32^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 2 | Yellow lesion | $49.48^{b}$ | $85.86^{c}$ | $42.48^{c}$ | $15.22^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 2 | Yellow lesion | $48.97^{b}$ | $85.31^{c}$ | $41.77^{c}$ | $15.20^{b}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 2 | Yellow lesion | $48.75^{c}$ | $87.52^{c}$ | $42.66^{c}$ | $15.58^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 2 | Yellow lesion | $46.73^{b}$ | $83.39^{c}$ | $40.99^{b}$ | $15.06^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 2 | Yellow lesion | $48.09^{b}$ | $84.07^{c}$ | $40.43^{b}$ | $15.06^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 2 | Yellow lesion | $48.53^{b}$ | $84.48^{c}$ | $40.99^{b}$ | $15.78^{b}$ | Yellowing,whitening and drying |
| 20 | Uma | 2 | Yellow lesion | $50.10^{b}$ | $86.05^{c}$ | $43.11^{d}$ | $16.02^{c}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**4.5.1.6. Parali isolate** (XPAI-3)

It was found that all the 19 varieties showed the CI values ranging from 45.81 to 49.87 and found susceptible to the bacterial leaf blight isolate (Table 5.6). The lesion length ranged from 15.20 cm to 20.70 cm. The variety Makaram showed the CI value of 1.22 and was found resistant to the isolate, with a lesion length of 1.10 cm. All the 19 varieties showed the initial symptom of yellow lesion in two days, whereas the variety Makaram took 15 days to produce the symptom.

**4.5.1.7. Pattambi isolate** (XPTB-4)

From the data presented in Table 5.7, it is evident that the varieties showed variation in resistance to Pattambi isolate of bacterial leaf blight pathogen. Among the 20 varieties, none of them were found completely free from infection. Nineteen varieties showed the CI values ranging from 31.58 to 38.67 and found moderately susceptible and showed the lesion length ranging from 12.96 to 14.82 cm. Makaram showed the CI value of 0.39 and found to be resistant to the isolate and showed the lesion length of 0.45 cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took 17 days to produce the symptom.

**4.5.1.8. Polpully isolate** (XPLY-5)

It was found that among the twenty varieties, none of them were found completely free from infection (Table 5.8). Nineteen varieties showed the CI values ranging from 39.20 to 46.62 and found susceptible to the bacterial blight isolate and showed the lesion length ranging from 15.18 to 17.78 cm. Makaram showed the CI value of 1.36 and was found to be resistant to the isolate and showed the lesion length of 1.30 cm. All the 19 varieties showed the initial symptom of yellow lesion in two days, whereas the variety Makaram took 15 days to produce the symptom.

**Table 5.6. Bacterial blight disease reaction of Parali isolate (XPAI-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 2 | Yellow lesion | $53.84^{b}$ | $89.54^{b}$ | $48.20^{b}$ | $19.96^{f}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 2 | Yellow lesion | $53.05^{b}$ | $89.84^{b}$ | $47.66^{b}$ | $19.98^{f}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 2 | Yellow lesion | $54.70^{d}$ | $90.47^{b}$ | $49.48^{c}$ | $20.30^{f}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 2 | Yellow lesion | $54.22^{c}$ | $91.99^{c}$ | $49.87^{d}$ | $20.70^{g}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 2 | Yellow lesion | $52.79^{b}$ | $90.83^{b}$ | $47.94^{b}$ | $20.28^{f}$ | Yellowing,whitening and drying |
| 6 | Harsha | 2 | Yellow lesion | $51.78^{b}$ | $90.81^{b}$ | $47.02^{b}$ | $19.36^{f}$ | Yellowing,whitening and drying |
| 7 | Jaya | 2 | Yellow lesion | $51.18^{b}$ | $90.17^{b}$ | $46.14^{b}$ | $20.18^{f}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 2 | Yellow lesion | $52.25^{b}$ | $90.46^{b}$ | $47.26^{b}$ | $19.86^{f}$ | Yellowing,whitening and drying |
| 9 | Kairali | 2 | Yellow lesion | $52.03^{b}$ | $90.35^{b}$ | $47.00^{b}$ | $17.28^{d}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 2 | Yellow lesion | $53.12^{b}$ | $90.21^{b}$ | $47.91^{b}$ | $16.38^{c}$ | Yellowing,whitening and drying |
| 11 | Karuna | 2 | Yellow lesion | $52.14^{b}$ | $90.47^{b}$ | $47.17^{b}$ | $16.34^{c}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 2 | Yellow lesion | $52.62^{b}$ | $90.82^{b}$ | $47.78^{b}$ | $15.34^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 15 | Yellow lesion | $4.51^{a}$ | $26.96^{a}$ | $1.21^{a}$ | $1.10^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 2 | Yellow lesion | $52.33^{b}$ | $90.74^{b}$ | $47.48^{b}$ | $15.22^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 2 | Yellow lesion | $51.86^{b}$ | $90.45^{b}$ | $46.90^{b}$ | $15.20^{b}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 2 | Yellow lesion | $51.04^{b}$ | $90.69^{b}$ | $46.28^{b}$ | $19.36^{f}$ | Yellowing,whitening and drying |
| 17 | Rohini | 2 | Yellow lesion | $53.41^{b}$ | $90.66^{b}$ | $48.42^{b}$ | $20.30^{f}$ | Yellowing,whitening and drying |
| 18 | Sabari | 2 | Yellow lesion | $52.98^{b}$ | $91.08^{b}$ | $48.25^{b}$ | $20.70^{g}$ | Yellowing,whitening and drying |
| 19 | Swetha | 2 | Yellow lesion | $51.03^{b}$ | $89.79^{b}$ | $45.81^{b}$ | $15.78^{b}$ | Yellowing,whitening and drying |
| 20 | Uma | 2 | Yellow lesion | $52.32^{b}$ | $90.71^{b}$ | $47.45^{b}$ | $18.94^{c}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.7. Bacterial blight disease reaction of Pattambi isolate (XPTB-4) on twenty cultivated rice varieties**

| Sl. No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $38.14^{b}$ | $87.63^{c}$ | $33.42^{b}$ | $13.04^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $40.93^{c}$ | $86.47^{c}$ | $35.39^{c}$ | $13.42^{b}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $12.96^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $44.33^{d}$ | $87.24^{c}$ | $38.67^{c}$ | $13.26^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $43.83^{d}$ | $87.48^{c}$ | $38.34^{c}$ | $14.52^{d}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $42.67^{c}$ | $87.55^{c}$ | $37.35^{d}$ | $14.82^{g}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $43.83^{d}$ | $87.48^{c}$ | $38.34^{c}$ | $14.66^{f}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $43.94^{d}$ | $86.86^{c}$ | $38.16^{d}$ | $14.40^{e}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $44.82^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.32^{e}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $41.94^{c}$ | $87.02^{c}$ | $36.49^{d}$ | $13.26^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $44.15^{d}$ | $86.09^{c}$ | $38.00^{d}$ | $14.64^{e}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $2.49^{a}$ | $16.01^{a}$ | $0.39^{a}$ | $0.45^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $44.33^{d}$ | $87.24^{c}$ | $38.66^{e}$ | $14.66^{f}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $44.82^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.32^{e}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $43.83^{d}$ | $87.48^{c}$ | $38.32^{e}$ | $14.32^{e}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $42.67^{c}$ | $87.55^{c}$ | $37.35^{d}$ | $13.88^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $41.94^{c}$ | $87.02^{c}$ | $36.49^{d}$ | $13.38^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $40.93^{c}$ | $86.47^{c}$ | $35.39^{c}$ | $14.40^{e}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $43.94^{d}$ | $86.86^{c}$ | $38.16^{d}$ | $14.30^{e}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.8. Bacterial blight disease reaction of Polpully isolate (XPLY-5) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 2 | Yellow lesion | 51.81$^{e}$ | 86.51$^{e}$ | 44.82$^{f}$ | 17.26$^{f}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 2 | Yellow lesion | 51.13$^{d}$ | 87.15$^{e}$ | 44.54$^{e}$ | 17.08$^{e}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 2 | Yellow lesion | 53.53$^{f}$ | 87.11$^{e}$ | 46.62$^{g}$ | 17.78$^{g}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 2 | Yellow lesion | 49.67$^{e}$ | 84.92$^{e}$ | 42.17$^{e}$ | 15.58$^{e}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 2 | Yellow lesion | 49.72$^{e}$ | 84.39$^{e}$ | 41.92$^{e}$ | 15.46$^{e}$ | Yellowing,whitening and drying |
| 6 | Harsha | 2 | Yellow lesion | 49.80$^{e}$ | 84.87$^{e}$ | 42.26$^{e}$ | 15.78$^{e}$ | Yellowing,whitening and drying |
| 7 | Jaya | 2 | Yellow lesion | 48.30$^{e}$ | 84.61$^{e}$ | 40.86$^{e}$ | 15.40$^{e}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 2 | Yellow lesion | 46.87$^{e}$ | 83.65$^{b}$ | 39.20$^{e}$ | 15.18$^{e}$ | Yellowing,whitening and drying |
| 9 | Kairali | 2 | Yellow lesion | 50.14$^{e}$ | 84.17$^{e}$ | 42.20$^{e}$ | 15.62$^{e}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 2 | Yellow lesion | 48.92$^{e}$ | 83.44$^{b}$ | 40.81$^{e}$ | 15.18$^{e}$ | Yellowing,whitening and drying |
| 11 | Karuna | 2 | Yellow lesion | 47.67$^{e}$ | 82.29$^{b}$ | 39.22$^{e}$ | 15.32$^{e}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 2 | Yellow lesion | 48.03$^{e}$ | 82.55$^{b}$ | 39.64$^{e}$ | 15.58$^{e}$ | Yellowing,whitening and drying |
| 13 | Makaram | 15 | Yellow lesion | 4.97$^{a}$ | 27.54$^{a}$ | 1.36$^{a}$ | 1.30$^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 2 | Yellow lesion | 47.22$^{e}$ | 83.63$^{b}$ | 39.49$^{e}$ | 15.18$^{e}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 2 | Yellow lesion | 49.53$^{e}$ | 83.91$^{e}$ | 41.56$^{e}$ | 15.32$^{e}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 2 | Yellow lesion | 49.46$^{e}$ | 85.69$^{d}$ | 42.38$^{e}$ | 15.98$^{e}$ | Yellowing,whitening and drying |
| 17 | Rohini | 2 | Yellow lesion | 48.11$^{e}$ | 82.47$^{b}$ | 39.67$^{e}$ | 15.40$^{e}$ | Yellowing,whitening and drying |
| 18 | Sabari | 2 | Yellow lesion | 49.67$^{e}$ | 84.92$^{e}$ | 42.17$^{e}$ | 15.46$^{e}$ | Yellowing,whitening and drying |
| 19 | Swetha | 2 | Yellow lesion | 50.14$^{e}$ | 84.17$^{e}$ | 42.20$^{e}$ | 15.78$^{e}$ | Yellowing,whitening and drying |
| 20 | Uma | 2 | Yellow lesion | 50.97$^{d}$ | 85.09$^{e}$ | 43.37$^{d}$ | 16.24$^{d}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**4.5.1.9. Akamala isolate** (XAKA-2)

As put in Table 5.9, nineteen varieties showed the CI values ranging from 31.95 to 37.28 and were found moderately susceptible to the bacterial leaf blight isolate, with lesion length ranging from 12.66 to 14.02 cm. Makaram showed the CI value of 0.80 and was found resistant to the isolate. The lesion length was 1.01 cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas Makaram took 17 days to produce the symptom.

**4.5.1.10. Kodakara isolate** (XKDA-1)

Regarding this isolate, it was found that 19 varieties showed the CI values ranging from 31.58 to 38.67 and were found moderately susceptible to the bacterial leaf blight isolate (Table 5.10). The lesion length ranged from 12.96 to 14.82 cm. Makaram showed the CI value of 0.39 and was found to be resistant to the isolate and showed the lesion length of 0.41cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took 17 days to produce the symptom.

**4.5.1.11. Mannuthy isolate** (XMTY-2)

As presented in Table 5.11., it was found that nineteen varieties showed the CI values ranging from 30.11 to 35.83 and found moderately susceptible to the bacterial leaf blight isolate and showed the lesion length ranging from 12.04 to 13.66 cm. Makaram showed the CI value of 0.58 and found to be resistant to the isolate and showed the lesion length of 1.16cm. All the nineteen varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took sixteen days to produce the symptom.

**4.5.1.12. Edathua isolate** (XEDA-3)

The results of pathogenic variability are presented in Table 5.12. From the data, it was found that among the 20 varieties, none of them were found completely free from disease.

**Table 5.10. Bacterial blight disease reaction of Kodakara isolate (XKDA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $38.14^{b}$ | $87.63^{c}$ | $33.42^{b}$ | $13.04^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $40.93^{c}$ | $86.47^{c}$ | $35.39^{c}$ | $13.42^{b}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $12.96^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $44.33^{d}$ | $87.25^{c}$ | $38.67^{e}$ | $13.26^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $44.33^{d}$ | $87.25^{c}$ | $38.67^{e}$ | $14.52^{d}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $43.95^{d}$ | $86.87^{c}$ | $38.17^{d}$ | $14.82^{f}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $14.66^{f}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $38.14^{b}$ | $87.63^{c}$ | $33.42^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $44.83^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.52^{d}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $14.30^{c}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $44.16^{d}$ | $86.09^{c}$ | $38.01^{d}$ | $14.64^{e}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $2.49^{a}$ | $16.01^{a}$ | $0.39^{a}$ | $0.41^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $12.96^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $44.83^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.32^{c}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $43.84^{d}$ | $87.48^{c}$ | $38.35^{e}$ | $14.32^{c}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $42.67^{c}$ | $87.56^{c}$ | $37.36^{d}$ | $13.88^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $41.94^{c}$ | $87.03^{c}$ | $36.50^{d}$ | $13.38^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $14.40^{c}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $43.95^{d}$ | $86.87^{c}$ | $38.17^{d}$ | $14.30^{c}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.9. Bacterial blight disease reaction of Akamala isolate (XAKA-2) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $42.00^{c}$ | $83.31^{b}$ | $34.99^{b}$ | $13.34^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $41.71^{b}$ | $84.64^{b}$ | $35.30^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $38.87^{b}$ | $82.21^{b}$ | $31.95^{b}$ | $12.82^{b}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $40.24^{b}$ | $83.78^{b}$ | $33.71^{b}$ | $12.80^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $41.16^{b}$ | $84.04^{b}$ | $34.59^{b}$ | $12.98^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $42.51^{c}$ | $82.77^{b}$ | $35.18^{b}$ | $13.26^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $40.62^{b}$ | $82.37^{b}$ | $33.45^{b}$ | $12.66^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $42.00^{c}$ | $83.32^{b}$ | $34.99^{b}$ | $13.36^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $42.62^{c}$ | $85.28^{c}$ | $36.34^{c}$ | $13.56^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $43.89^{d}$ | $84.96^{b}$ | $37.28^{c}$ | $13.66^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $41.11^{b}$ | $83.77^{b}$ | $34.43^{b}$ | $12.78^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $41.76^{b}$ | $83.78^{b}$ | $34.98^{b}$ | $13.22^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $3.77^{a}$ | $21.38^{a}$ | $0.80^{a}$ | $1.01^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $40.42^{b}$ | $83.95^{b}$ | $33.93^{b}$ | $13.02^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $41.02^{b}$ | $82.68^{b}$ | $33.93^{b}$ | $12.80^{b}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $40.98^{b}$ | $83.97^{b}$ | $34.41^{b}$ | $13.02^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $41.16^{b}$ | $83.83^{b}$ | $34.50^{b}$ | $12.90^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $42.48^{c}$ | $83.21^{b}$ | $35.34^{c}$ | $13.28^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $44.20^{c}$ | $84.00^{b}$ | $37.12^{d}$ | $14.02^{d}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $42.03^{c}$ | $83.96^{b}$ | $35.28^{b}$ | $13.82^{c}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.10. Bacterial blight disease reaction of Kodakara isolate (XKDA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21 DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $38.14^{b}$ | $87.63^{c}$ | $33.42^{b}$ | $13.04^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $40.93^{c}$ | $86.47^{c}$ | $35.39^{c}$ | $13.42^{b}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $12.96^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $44.33^{d}$ | $87.25^{c}$ | $38.67^{e}$ | $13.26^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $44.33^{d}$ | $87.25^{c}$ | $38.67^{e}$ | $14.52^{d}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $43.95^{d}$ | $86.87^{c}$ | $38.17^{d}$ | $14.82^{f}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $14.66^{f}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $38.14^{b}$ | $87.63^{c}$ | $33.42^{b}$ | $13.00^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $44.83^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.52^{d}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $14.30^{c}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $44.16^{d}$ | $86.09^{c}$ | $38.01^{d}$ | $14.64^{e}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $2.49^{a}$ | $16.01^{a}$ | $0.39^{a}$ | $0.41^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $38.31^{b}$ | $82.44^{b}$ | $31.58^{b}$ | $12.96^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $44.83^{e}$ | $85.70^{c}$ | $38.41^{e}$ | $14.32^{c}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $43.84^{d}$ | $87.48^{c}$ | $38.35^{e}$ | $14.32^{c}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $42.67^{c}$ | $87.56^{c}$ | $37.36^{d}$ | $13.88^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $41.94^{c}$ | $87.03^{c}$ | $36.50^{d}$ | $13.38^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $37.46^{b}$ | $86.59^{c}$ | $32.43^{b}$ | $14.40^{c}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $43.95^{d}$ | $86.87^{c}$ | $38.17^{d}$ | $14.30^{c}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.11. Bacterial blight disease reaction of Mannuthy isolate (XMTY-2) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $39.53^{b}$ | $84.04^{d}$ | $33.22^{b}$ | $12.62^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $37.03^{b}$ | $81.05^{b}$ | $30.11^{b}$ | $12.58^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $42.44^{e}$ | $83.36^{b}$ | $35.37^{d}$ | $13.44^{d}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $41.22^{d}$ | $85.57^{e}$ | $35.27^{d}$ | $13.35^{d}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $40.88^{e}$ | $84.00^{d}$ | $34.33^{c}$ | $13.16^{c}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $41.09^{d}$ | $84.51^{d}$ | $34.72^{c}$ | $12.92^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $37.18^{b}$ | $83.82^{b}$ | $31.16^{b}$ | $12.34^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $37.45^{b}$ | $84.14^{d}$ | $31.51^{b}$ | $12.22^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $39.36^{b}$ | $81.95^{b}$ | $32.25^{b}$ | $12.66^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $40.18^{b}$ | $83.72^{b}$ | $33.63^{b}$ | $12.26^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $39.88^{b}$ | $84.70^{d}$ | $33.77^{b}$ | $12.93^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $37.79^{b}$ | $80.88^{b}$ | $30.56^{b}$ | $12.04^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 16 | Yellow lesion | $2.86^{a}$ | $20.53^{a}$ | $0.58^{a}$ | $1.16^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $38.28^{b}$ | $82.57^{b}$ | $31.60^{b}$ | $12.08^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $41.36^{d}$ | $82.58^{b}$ | $34.15^{b}$ | $12.74^{b}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $41.23^{d}$ | $82.98^{b}$ | $34.21^{b}$ | $12.86^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $39.86^{b}$ | $83.09^{b}$ | $33.11^{b}$ | $12.28^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $40.79^{c}$ | $82.93^{b}$ | $33.82^{b}$ | $12.63^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $43.05^{f}$ | $83.25^{b}$ | $35.83^{d}$ | $13.38^{d}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $41.40^{d}$ | $84.16^{d}$ | $35.02^{d}$ | $13.66^{e}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

Nineteen varieties showed the CI values ranging from 34.13 to 38.90 and were found moderately susceptible to the bacterial leaf blight isolate. The lesion length ranged from 13.42 to 14.94 cm. Makaram showed the CI value of 0.65 and was found to be resistant to the isolate and showed the lesion length of 0.84 cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took 17 days to produce the symptom.

**4.5.1.13. Karuvatta isolate** (XKVA-1)

As presented in Table 5.13, regarding Karuvatta isolate, it was found that 19 varieties showed the CI values ranging from 26.88 to 37.74 and found moderately susceptible to the bacterial leaf blight isolate and showed the lesion length ranging from 11.44 to 14.50 cm. Makaram showed the CI value of 0.85 and found to be highly resistant to the isolate and showed the lesion length of 0.73cm. All the 19 varieties showed the initial symptom of yellow lesion in three days, whereas the variety Makaram took 17 days to produce the symptom.

**4.5.1.14. Moncombu isolate** (XMOU-1)

Presented in Table 5.14, it was found that 19 varieties showed the CI values ranging from 9.95 to 11.43 and found resistant to moderately resistant to the bacterial leaf blight isolate. The lesion length ranged from 5.24 cm to 6.74 cm. Makaram showed the CI value of 0.58 and was found resistant to the isolate and showed the lesion length of 0.4 cm. All the 19 varieties showed the initial symptom of yellow lesion in five days, whereas the variety Makaram had taken 20 days to produce the symptom.

Cumulative results of the pathogenic variability studies of the 14 isolates on 20 rice varieties are presented in the Table 6. The study revealed that the isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed susceptible reaction on nineteen varieties. The seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) showed moderately susceptible

**Table 5.12. Bacterial blight disease reaction of Edathua isolate (XEDA-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $41.49^{b}$ | $82.98^{b}$ | $34.42^{b}$ | $13.98^{b}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $41.60^{b}$ | $82.06^{b}$ | $34.13^{b}$ | $13.42^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $43.83^{b}$ | $84.77^{b}$ | $37.15^{b}$ | $14.30^{b}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $43.34^{b}$ | $84.77^{b}$ | $36.73^{b}$ | $13.50^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $44.36^{b}$ | $83.33^{b}$ | $36.96^{b}$ | $14.02^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $43.57^{b}$ | $85.19^{c}$ | $37.11^{b}$ | $13.58^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $44.98^{b}$ | $84.80^{b}$ | $38.14^{b}$ | $14.26^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $41.60^{b}$ | $82.06^{b}$ | $34.13^{b}$ | $14.20^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $42.63^{b}$ | $84.69^{b}$ | $36.10^{b}$ | $13.66^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $41.75^{b}$ | $84.96^{c}$ | $35.47^{b}$ | $13.62^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $44.58^{b}$ | $83.86^{b}$ | $37.38^{b}$ | $14.20^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $44.30^{b}$ | $84.27^{b}$ | $37.33^{b}$ | $14.04^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $3.22^{a}$ | $20.23^{a}$ | $0.65^{a}$ | 0.84a | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $43.75^{b}$ | $87.33^{c}$ | $38.20^{b}$ | $14.94^{b}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $43.34^{b}$ | $84.77^{b}$ | $36.73^{b}$ | $13.50^{b}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $44.36^{b}$ | $83.33^{b}$ | $36.96^{b}$ | $13.98^{b}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $42.48^{b}$ | $84.02^{b}$ | $35.69^{b}$ | $14.68^{b}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $45.24^{b}$ | $85.99^{d}$ | $38.90^{d}$ | $14.26^{b}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $45.19^{b}$ | $85.39^{c}$ | $38.58^{b}$ | $14.02^{b}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $41.75^{b}$ | $84.96^{c}$ | $35.47^{b}$ | $14.30^{b}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.13. Bacterial blight disease reaction of Karuvatta isolate (XKVA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lesion length (cm) | |
| 1 | Aiswarya | 3 | Yellow lesion | $36.69^{b}$ | $80.01^{b}$ | $29.35^{b}$ | $13.18^{f}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 3 | Yellow lesion | $41.45^{f}$ | $83.11^{e}$ | $34.44^{f}$ | $14.50^{g}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 3 | Yellow lesion | $40.53^{e}$ | $83.25^{e}$ | $37.74^{e}$ | $13.88^{f}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 3 | Yellow lesion | $38.33^{c}$ | $81.84^{c}$ | $31.36^{c}$ | $13.44^{f}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 3 | Yellow lesion | $39.04^{d}$ | $81.75^{b}$ | $31.91^{c}$ | $13.00^{e}$ | Yellowing,whitening and drying |
| 6 | Harsha | 3 | Yellow lesion | $37.79^{c}$ | $80.28^{b}$ | $30.33^{b}$ | $12.56^{c}$ | Yellowing,whitening and drying |
| 7 | Jaya | 3 | Yellow lesion | $36.97^{b}$ | $81.26^{b}$ | $30.04^{b}$ | $12.06^{b}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 3 | Yellow lesion | $33.83^{b}$ | $79.48^{b}$ | $26.88^{b}$ | $11.56^{b}$ | Yellowing,whitening and drying |
| 9 | Kairali | 3 | Yellow lesion | $34.84^{b}$ | $81.15^{b}$ | $28.27^{b}$ | $11.44^{b}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 3 | Yellow lesion | $36.73^{b}$ | $81.00^{b}$ | $29.75^{b}$ | $11.88^{b}$ | Yellowing,whitening and drying |
| 11 | Karuna | 3 | Yellow lesion | $35.95^{b}$ | $80.68^{b}$ | $29.00^{b}$ | $11.72^{b}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 3 | Yellow lesion | $36.27^{b}$ | $80.58^{b}$ | $29.22^{b}$ | $11.54^{b}$ | Yellowing,whitening and drying |
| 13 | Makaram | 17 | Yellow lesion | $3.92^{a}$ | $21.90^{a}$ | $0.85^{a}$ | $0.73^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 3 | Yellow lesion | $36.47^{b}$ | $80.45^{b}$ | $29.34^{b}$ | $12.72^{d}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 3 | Yellow lesion | $36.40^{b}$ | $80.71^{b}$ | $29.37^{b}$ | $13.06^{e}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 3 | Yellow lesion | $36.98^{b}$ | $80.76^{b}$ | $29.86^{b}$ | $13.14^{f}$ | Yellowing,whitening and drying |
| 17 | Rohini | 3 | Yellow lesion | $35.34^{b}$ | $79.91^{b}$ | $28.24^{b}$ | $13.86^{g}$ | Yellowing,whitening and drying |
| 18 | Sabari | 3 | Yellow lesion | $39.22^{d}$ | $82.31^{d}$ | $32.28^{d}$ | $13.06^{e}$ | Yellowing,whitening and drying |
| 19 | Swetha | 3 | Yellow lesion | $38.45^{c}$ | $81.60^{b}$ | $31.37^{c}$ | $13.00^{e}$ | Yellowing,whitening and drying |
| 20 | Uma | 3 | Yellow lesion | $41.56^{g}$ | $81.10^{b}$ | $33.70^{e}$ | $13.42^{f}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 5.14. Bacterial blight disease reaction of Moncombu isolate (XMOU-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | Initial symptom expression | **21DAI | | | | Symptom expression |
|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Lession length (cm) | |
| 1 | Aiswarya | 5 | Yellow lesion | $19.99^{e}$ | $54.74^{e}$ | $10.94^{d}$ | $6.02^{c}$ | Yellowing,whitening and drying |
| 2 | Anashwara | 5 | Yellow lesion | $18.44^{c}$ | $53.99^{e}$ | $9.95^{c}$ | $5.68^{b}$ | Yellowing,whitening and drying |
| 3 | Annapoorna | 5 | Yellow lesion | $14.83^{b}$ | $48.49^{b}$ | $10.34^{c}$ | $5.86^{c}$ | Yellowing,whitening and drying |
| 4 | Aswathi | 5 | Yellow lesion | $16.85^{b}$ | $51.09^{b}$ | $9.98^{c\,b}$ | $5.38^{b}$ | Yellowing,whitening and drying |
| 5 | Bhadra | 5 | Yellow lesion | $16.78^{b}$ | $51.83^{b}$ | $10.01^{c}$ | $5.24^{b}$ | Yellowing,whitening and drying |
| 6 | Harsha | 5 | Yellow lesion | $16.89^{b}$ | $52.68^{b}$ | $10.43^{c}$ | $5.44^{b}$ | Yellowing,whitening and drying |
| 7 | Jaya | 5 | Yellow lesion | $18.34^{c}$ | $54.12^{e}$ | $9.92^{c}$ | $6.00^{c}$ | Yellowing,whitening and drying |
| 8 | Jyothi | 5 | Yellow lesion | $18.66^{c}$ | $55.01^{e}$ | $10.26^{c}$ | $6.06^{c}$ | Yellowing,whitening and drying |
| 9 | Kairali | 5 | Yellow lesion | $18.50^{c}$ | $56.46^{d}$ | $10.44^{d}$ | $6.02^{c}$ | Yellowing,whitening and drying |
| 10 | Kanchana | 5 | Yellow lesion | $18.21^{c}$ | $54.69^{e}$ | $9.95^{c}$ | $5.98^{c}$ | Yellowing,whitening and drying |
| 11 | Karuna | 5 | Yellow lesion | $19.97^{e}$ | $57.22^{e}$ | $11.42^{f}$ | $6.24^{d}$ | Yellowing,whitening and drying |
| 12 | Kunju Kunju Varna | 5 | Yellow lesion | $18.64^{c}$ | $55.99^{e}$ | $10.43^{c}$ | $6.08^{c}$ | Yellowing,whitening and drying |
| 13 | Makaram | 20 | Yellow lesion | $2.92^{a}$ | $19.90^{a}$ | $0.58^{a}$ | $0.40^{a}$ | Yellowing,whitening and drying |
| 14 | Manupriya | 5 | Yellow lesion | $18.75^{c}$ | $55.35^{e}$ | $10.37^{c}$ | $6.74^{e}$ | Yellowing,whitening and drying |
| 15 | Matta Triveni | 5 | Yellow lesion | $18.25^{c}$ | $54.88^{e}$ | $10.01^{c}$ | $5.88^{c}$ | Yellowing,whitening and drying |
| 16 | Neeraja | 5 | Yellow lesion | $18.16^{c}$ | $54.99^{e}$ | $9.98^{c}$ | $5.86^{c}$ | Yellowing,whitening and drying |
| 17 | Rohini | 5 | Yellow lesion | $18.29^{c}$ | $55.37^{e}$ | $10.94^{c}$ | $5.92^{c}$ | Yellowing,whitening and drying |
| 18 | Sabari | 5 | Yellow lesion | $19.33^{d}$ | $56.64^{d}$ | $10.94^{d}$ | $6.26^{d}$ | Yellowing,whitening and drying |
| 19 | Swetha | 5 | Yellow lesion | $18.66^{c}$ | $55.46^{e}$ | $10.34^{c}$ | $6.16^{c}$ | Yellowing,whitening and drying |
| 20 | Uma | 5 | Yellow lesion | $19.36^{d}$ | $58.19^{f}$ | $11.26^{e}$ | $6.24^{d}$ | Yellowing,whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

## Table 6. Pathotype study of *Xoo* isolates on selected rice varieties

| Varieties / Isolates | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ | $V_8$ | $V_9$ | $V_{10}$ | $V_{11}$ | $V_{12}$ | $V_{13}$ | $V_{14}$ | $V_{15}$ | $V_{16}$ | $V_{17}$ | $V_{18}$ | $V_{19}$ | $V_{20}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XAMI-3 | S | S | S | S | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S |
| XERM-1 | S | S | S | S | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S |
| XKOR-3 | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | R | MR | MR | MR | MR | MR | MR | MR |
| XMRA-2 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | R | MS | MS | MS | MS | MS | MS | MS |
| XNRA-1 | S | S | S | S | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S |
| XPAI-3 | S | S | S | S | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S |
| XPTB-4 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XPLY-5 | S | S | S | S | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S |
| XAGA-2 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XKDA-1 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XMTY-2 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XEDA-3 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XKVA-1 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | R | MS | MS | MS | MS | MS | MS | MS |
| XMOU-1 | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | MR | R | MR | MR | MR | MR | MR | MR | MR |

$V_1$. Aiswarya $V_2$. Anashwara $V_3$. Annapoorna $V_4$. Aswathi $V_5$. Bhadra $V_6$. Harsha $V_7$. Jaya $V_8$. Jyothi $V_9$. Kairali $V_{10}$. Kanchana $V_{11}$. Karuna $V_{12}$. Kunju Kunju Varna $V_{13}$. Makaram $V_{14}$. Manupriya $V_{15}$. Matta Triveni $V_{16}$. Neeraja $V_{17}$. Rohini $V_{18}$. Sabari $V_{19}$. Swetha $V_{20}$. Uma

R- Resistant MR - Moderately Resistant MS- Moderayely Susceptible R- Resistant

reaction on 19 rice varieties. The two isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed moderately resistant reaction on 19 rice varieties. All the fourteen isolates showed resistant reaction to the variety Makaram.

Reaction of the variety Makaram to 14 isolates of *Xoo* is presented in Table 7. The 14 isolates of *Xoo* showed the PDI, PDS and CI values ranging from 0.15 - 6.23, 1.43 – 34.77 and 0.21 – 2.16 respectively on the variety Makaram. The lesion length ranged from 0.40 – 1.92 cm. The isolates showed the initial symptom of yellow lesion at 15 to 20 days interval. Based on these observations, it was clear that the variety Makaram is identified as resistant to all the 14 isolates of *Xoo*.

### 4.5.2. Pathogenic variability of *Xoo* isolates on near isogenic lines and rice differentials

The pathogenic variability of 14 isolates obtained from three districts were studied on six near isogenic lines and three rice differentials as mentioned in the materials and methods.

#### 4.5.2.1. Athimani isolate (XAMI-3)

The disease reaction of Athimani isolate is presented in Table 8.1. The near isogenic line IRBB-60 showed the CI value of 7.94 and found to be resistant to the pathogen and showed the lesion length of 4.54 cm. The other isogenc lines showed the CI values ranged from 10.90 to 22.52 and found to be moderately susceptible to the pathogen and showed the lesion length ranged from 9.20 cm to 11.50 cm. The rice differentials showed the CI values ranged from 41.68 to 42.23 were found to be susceptible to the pathogen. They showed the lesion length ranged from 15.18 cm to 16.52 cm. It was found that the initial symptom of yellow lesion on the cut ends was noticed on second day after inoculation on six near isogenic lines and on three rice differentials.

#### 4.5.2.2. Erattakulam isolate (XERM-1)

As presented in Table 8.2, the near isogenic line IRBB-60 showed the CI value of 4.52 and found to be resistant to the pathogen and showed the lesion length of 4.70 cm. The

Table 7. Bacterial blight disease reaction of Makaram variety to fourteen isolates of *Xoo*

| Sl.No. | Isolates | *Incubation period (days) | Initial symptom expression | 21 DAI | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Disease reaction | Lesion length (cm) | Disease reaction |
| 1 | Athimani (XAMI-3 ) | 15 | Yellow lesion | 6.23 | 34.77 | 2.16 | R | 1.92 | R |
| 2 | Erattakulam (XERM-1) | 15 | Yellow lesion | 4.76 | 26.19 | 1.04 | R | 1.16 | R |
| 3 | Kodallur (XKOR-3) | 20 | Yellow lesion | 0.15 | 1.43 | 0.21 | R | 0.40 | R |
| 4 | Manchira (XMRA-2) | 16 | Yellow lesion | 3.66 | 22.25 | 0.81 | R | 0.64 | R |
| 5 | Nenmara (XNRA-1) | 15 | Yellow lesion | 4.53 | 28.32 | 1.28 | R | 1.32 | R |
| 6 | Parali (XPAI-3) | 15 | Yellow lesion | 4.51 | 26.96 | 1.21 | R | 1.10 | R |
| 7 | Pattambi (XPTB-4 ) | 17 | Yellow lesion | 2.49 | 16.01 | 0.39 | R | 0.45 | R |
| 8 | Polpully (XPLY-5) | 15 | Yellow lesion | 4.97 | 27.54 | 1.36 | R | 1.30 | R |
| 9 | Akamala (XAKA-2) | 17 | Yellow lesion | 3.77 | 21.38 | 0.80 | R | 1.01 | R |
| 10 | Kodakara (XKDA-1) | 17 | Yellow lesion | 2.49 | 16.01 | 0.39 | R | 0.41 | R |
| 11 | Mannuthy (XMTY-2) | 16 | Yellow lesion | 2.86 | 20.53 | 0.58 | R | 1.16 | R |
| 12 | Edathua (XEDA-3) | 17 | Yellow lesion | 3.22 | 20.23 | 0.65 | R | 0.84 | R |
| 13 | Karuvatta (XKVA-1) | 17 | Yellow lesion | 3.92 | 21.90 | 0.85 | R | 0.73 | R |
| 14 | Moncombu (XMOU-1) | 20 | Yellow lesion | 2.92 | 9.90 | 0.58 | R | 0.40 | R |

* Incubation period refers to the time taken (days) for expression of initial symptom

R- Resistant

**Table 8.1. Bacterial blight disease reaction of Athimani isolate (XAMI-3) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 2 | Yellow lesion | 28.21[b] | 71.17[c] | 20.07[b] | 10.22[b] | Yellowing, whitening and drying |
| IRBB -5 | 2 | Yellow lesion | 28.21[b] | 71.74[c] | 20.23[b] | 11.50[c] | Yellowing, whitening and drying |
| IRBB -13 | 2 | Yellow lesion | 30.51[b] | 73.83[d] | 22.52[c] | 10.00[b] | Yellowing, whitening and drying |
| IRBB -21 | 2 | Yellow lesion | 28.21[b] | 71.74[c] | 20.23[b] | 10.34[b] | Yellowing, whitening and drying |
| IRBB -57 | 2 | Yellow lesion | 19.21[a] | 56.79[a] | 10.90[a] | 9.20[a] | Yellowing, whitening and drying |
| IRBB- 60 | 2 | Yellow lesion | 15.78[a] | 50.38[a] | 7.94[a] | 4.54[a] | Yellowing, whitening and drying |
| Ajaya | 2 | Yellow lesion | 42.88[c] | 95.18[e] | 40.81[d] | 15.28[d] | Yellowing, whitening and drying |
| IR-8 | 2 | Yellow lesion | 43.72[c] | 96.60[e] | 42.23[e] | 16.52[e] | Yellowing, whitening and drying |
| IR-24 | 2 | Yellow lesion | 43.80[c] | 95.18[e] | 41.68[e] | 15.18[d] | Yellowing, whitening and drying |

**Table 8.2. Bacterial blight disease reaction of Erattakulam isolate (XERM-1) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 2 | Yellow lesion | 38.42[c] | 83.41[c] | 32.04[d] | 11.06[b] | Yellowing, whitening and drying |
| IRBB -5 | 2 | Yellow lesion | 36.02[c] | 76.90[b] | 27.69[c] | 10.12[b] | Yellowing, whitening and drying |
| IRBB -13 | 2 | Yellow lesion | 37.42[c] | 76.92[b] | 28.78[c] | 10.26[b] | Yellowing, whitening and drying |
| IRBB -21 | 2 | Yellow lesion | 41.03[d] | 84.04[c] | 34.48[e] | 11.76[c] | Yellowing, whitening and drying |
| IRBB -57 | 2 | Yellow lesion | 29.98[b] | 73.13[a] | 21.92[b] | 8.68[a] | Yellowing, whitening and drying |
| IRBB- 60 | 2 | Yellow lesion | 26.56[a] | 69.02[a] | 4.52[a] | 4.70[a] | Yellowing, whitening and drying |
| Ajaya | 2 | Yellow lesion | 44.76[e] | 89.41[d] | 40.01[f] | 15.02[d] | Yellowing, whitening and drying |
| IR-8 | 2 | Yellow lesion | 45.21[e] | 90.73[d] | 41.01[f] | 15.34[d] | Yellowing, whitening and drying |
| IR-24 | 2 | Yellow lesion | 45.56[e] | 90.72[d] | 41.33[f] | 15.78[d] | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

other isogenc lines showed the CI values ranged from 21.92 to 34.48 and found to be moderately susceptible to the pathogen and showed the lesion length ranged from 8.68 cm to 11.76 cm. The rice differentials showed the CI values ranged from 40.61 to 41.33 found to be susceptible to the pathogen. They showed the lesion length ranged from 15.02 cm to 15.78 cm. Yellow lesions on the cut ends was noticed as initial symptom, on second day after inoculation on six near isogenic lines and on three rice differentials.

**4.5.2.3. Kodallur isolate** (XKOR-3)

As mentioned in Table 8.3, the near isogenic lines and rice differentials showed CI values ranged from 0.58 to 6.34 found to be resistant to the pathogen.The lesion length ranged from 0.50 cm to 3.76 cm. Initial symptom of yellow lesion on the cut ends was noticed on seventh day after inoculation in all the cases.

**4.5.2.4. Manchira isolate** (XMRA-2)

As seen in Table 8.4, the near isogenic line IRBB-60 showed the CI value of 4.71 and lesion length of 3.42 cm found to be resistant to the pathogen. The other isogenc lines showed the CI values ranged from 9.65 to 18.65 and lesion length ranged from 5.38 cm to 7.70 cm found to be moderately resistant to the pathogen. The rice differentials showed the CI values ranged from 30.83 to 43.25 found to be moderately susceptible to the pathogen. They showed the lesion length ranged from 10.96 cm to 11.96 cm. The initial symptom as yellow lesion appeared on third day after inoculation on near isogenic lines and on rice differentials.

**4.5.2.5. Nenmara isolate** (XNRA-1)

The disease reaction of the Nenmara isolate is presented in Table 8.5.The disease reaction showed that the near isogenic line IRBB-60 was found resistant with the CI value of 8.37. It showed the lesion length of 4.82 cm. The other isogenc lines showed the CI values ranged from 19.55 to 29.08 and the lesion length ranged from 7.76 cm to 10.46 cm found to be moderately susceptible to the pathogen. The rice differentials showed the CI values ranged from 42.07 to 43.26 and lesion length ranged from 15.60 cm to 15.98 cm found to be

**Table 8.3. Bacterial blight disease reaction of Kodallur isolate (XKOR-3) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 7 | Yellow lesion | 7.96[a] | 37.14[b] | 2.95[b] | 2.02[b] | Yellowing, whitening and drying |
| IRBB -5 | 7 | Yellow lesion | 8.19[b] | 33.15[b] | 2.71[b] | 1.94[b] | Yellowing, whitening and drying |
| IRBB -13 | 7 | Yellow lesion | 8.06[b] | 38.14[b] | 3.07[b] | 2.26[b] | Yellowing, whitening and drying |
| IRBB -21 | 7 | Yellow lesion | 7.44[b] | 33.05[b] | 2.45[b] | 1.60[b] | Yellowing, whitening and drying |
| IRBB -57 | 7 | Yellow lesion | 4.59[a] | 21.30[a] | 0.97[a] | 0.62[a] | Yellowing, whitening and drying |
| IRBB- 60 | 7 | Yellow lesion | 3.23[a] | 18.20[a] | 0.58[a] | 0.50[a] | Yellowing, whitening and drying |
| Ajaya | 7 | Yellow lesion | 13.31[c] | 43.17[c] | 5.74[c] | 3.48[c] | Yellowing, whitening and drying |
| IR-8 | 7 | Yellow lesion | 13.31[c] | 43.18[c] | 5.74[c] | 3.54[c] | Yellowing, whitening and drying |
| IR-24 | 7 | Yellow lesion | 13.96[c] | 45.42[d] | 6.34[c] | 3.76[c] | Yellowing, whitening and drying |

**Table 8.4. Bacterial blight disease reaction of Manchira isolate (XMRA-2) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 20.07[a] | 57.38[a] | 11.51[b] | 6.02[a] | Yellowing, whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 20.57[a] | 57.23[a] | 11.77[b] | 6.20[a] | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 25.81[b] | 70.85[b] | 18.28[b] | 7.56[c] | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 26.19[b] | 71.22[b] | 18.65[b] | 7.70[c] | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 18.36[a] | 52.56[a] | 9.65[a] | 5.38[b] | Yellowing, whitening and drying |
| IRBB- 60 | 3 | Yellow lesion | 11.25[a] | 41.92[a] | 4.71[a] | 3.42[a] | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 37.42[c] | 82.39[c] | 30.83[c] | 10.96[c] | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 49.29[c] | 85.72[c] | 42.25[d] | 11.54[c] | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 50.04[c] | 86.44[d] | 43.25[d] | 11.96[c] | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

**Table 8.5. Bacterial blight disease reaction of Nenmara isolate (XNRA-1) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 2 | Yellow lesion | 35.60$^{d}$ | 81.69$^{e}$ | 29.08$^{e}$ | 10.46$^{e}$ | Yellowing, whitening and drying |
| IRBB -5 | 2 | Yellow lesion | 32.91$^{c}$ | 84.28$^{e}$ | 27.73$^{e}$ | 10.10$^{e}$ | Yellowing, whitening and drying |
| IRBB -13 | 2 | Yellow lesion | 33.19$^{e}$ | 81.55$^{e}$ | 27.06$^{e}$ | 10.02$^{e}$ | Yellowing, whitening and drying |
| IRBB -21 | 2 | Yellow lesion | 33.87$^{e}$ | 79.95$^{e}$ | 27.07$^{e}$ | 10.05$^{e}$ | Yellowing, whitening and drying |
| IRBB -57 | 2 | Yellow lesion | 26.51$^{b}$ | 73.77$^{b}$ | 19.55$^{b}$ | 7.76$^{b}$ | Yellowing, whitening and drying |
| IRBB- 60 | 2 | Yellow lesion | 20.57$^{a}$ | 40.71$^{a}$ | 8.37$^{a}$ | 4.82$^{a}$ | Yellowing, whitening and drying |
| Ajaya | 2 | Yellow lesion | 49.50$^{e}$ | 84.99$^{d}$ | 42.07$^{d}$ | 15.60$^{d}$ | Yellowing, whitening and drying |
| IR-8 | 2 | Yellow lesion | 49.23$^{e}$ | 87.24$^{e}$ | 42.94$^{d}$ | 15.78$^{d}$ | Yellowing, whitening and drying |
| IR-24 | 2 | Yellow lesion | 49.41$^{e}$ | 87.57$^{e}$ | 43.26$^{d}$ | 15.98$^{d}$ | Yellowing, whitening and drying |

**Table 8.6. Bacterial blight disease reaction of Parali isolate (XPAI-3) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 2 | Yellow lesion | 35.12$^{c}$ | 84.28$^{e}$ | 29.59$^{e}$ | 10.58$^{e}$ | Yellowing, whitening and drying |
| IRBB -5 | 2 | Yellow lesion | 35.89$^{b}$ | 85.99$^{e}$ | 30.86$^{e}$ | 10.90$^{e}$ | Yellowing, whitening and drying |
| IRBB -13 | 2 | Yellow lesion | 36.21$^{b}$ | 84.14$^{e}$ | 30.46$^{e}$ | 10.78$^{e}$ | Yellowing, whitening and drying |
| IRBB -21 | 2 | Yellow lesion | 34.44$^{b}$ | 83.48$^{e}$ | 28.75$^{e}$ | 10.40$^{e}$ | Yellowing, whitening and drying |
| IRBB -57 | 2 | Yellow lesion | 31.37$^{b}$ | 77.00$^{b}$ | 24.15$^{b}$ | 9.24$^{b}$ | Yellowing, whitening and drying |
| IRBB- 60 | 2 | Yellow lesion | 20.94$^{a}$ | 40.37$^{a}$ | 8.45$^{a}$ | 4.60$^{a}$ | Yellowing, whitening and drying |
| Ajaya | 2 | Yellow lesion | 48.85$^{e}$ | 85.23$^{e}$ | 41.72$^{d}$ | 15.44$^{d}$ | Yellowing, whitening and drying |
| IR-8 | 2 | Yellow lesion | 49.27$^{e}$ | 89.89$^{e}$ | 44.28$^{d}$ | 16.14$^{e}$ | Yellowing, whitening and drying |
| IR-24 | 2 | Yellow lesion | 48.96$^{e}$ | 91.24$^{e}$ | 44.57$^{d}$ | 16.44$^{e}$ | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

susceptible to the pathogen. Yellow lesion as initial symptom on the cut ends was noticed on second day after inoculation in all the nine cases.

**4.5.2.6. Parali isolate** (XPAI-3)

As results presented in Table 8.6, the Parali isolate, showed CI value of 8.45, lesion length of 4.60 cm on IRBB-60 found resistant to the pathogen. On the other isogenc lines, it showed the CI values ranged from 24.15 to 30.86 and lesion length ranged from 9.24 cm to 10.90 cm found to be moderately susceptible to the pathogen. The rice differentials showed the CI values ranged from 41.72 to 44.57 and the lesion length ranged from 15.44 cm to 16.44 cm found to be susceptible to the pathogen. The isolate took two days to show the initial symptom of yellow lesion on the cut ends in all the cases.

**4.5.2.7. Pattambi isolate** (XPTB-4)

As seen in Table 8.7, the Pattambi isolate showed the CI value of 8.27, lesion length of 4.44 cm on the near isogenic line IRBB-60 and found to be resistant to the pathogen. The other isogenc lines showed the CI values ranged from 12.46 to 18.89, lesion length ranged from 5.96 cm to 7.86 cm and found to be moderately resistant to the pathogen. The rice differentials showed the CI values ranged from 21.56 to 42.72, lesion length ranged from 10.35 cm to 11.52 cm and found to be moderately susceptible to the pathogen.Yellow lesion as the initial symptom on the cut ends was noticed on third day after inoculation on six near isogenic lines and three rice differentials.

**4.5.2.8. Polpully isolate** (XPLY-5)

As put in Table 8.8, the Polpully isolate, showed the CI value of 8.12 and lesion length of 4.14 cm on IRBB-60 found resistant to the pathogen. The other isogenc lines showed the CI values ranged from 22.85 to 32.72 and the lesion length ranged from 10.20 cm to 11.38 cm, found moderately susceptible to the pathogen. The rice differentials showed the CI values ranged from 39.06 to 39.21 and lesion length ranged from 15.10 cm to 15.26 cm found susceptible to the pathogen. Initial symptom of yellow lesions on the cut ends of all the cases

**Table 8.7. Bacterial blight disease reaction of Pattambi isolate (XPTB-4) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 24.51[a] | 62.58[a] | 15.33[b] | 6.18[b] | Yellowing, whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 21.36[a] | 60.26[a] | 16.08[b] | 6.52[b] | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 23.07[a] | 73.23[a] | 16.96[b] | 7.38[b] | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 24.67[a] | 76.55[b] | 18.89[b] | 7.86[b] | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 20.54[a] | 59.50[a] | 12.46[a] | 5.96[a] | Yellowing, whitening and drying |
| IRBB- 60 | 3 | Yellow lesion | 16.07[a] | 49.87[a] | 8.27[a] | 4.44[a] | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 24.98[a] | 86.32[c] | 21.56[c] | 10.35[c] | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 43.98[b] | 87.32[c] | 38.40[d] | 11.20[c] | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 48.83[b] | 87.50[c] | 42.72[d] | 11.52[c] | Yellowing, whitening and drying |

**Table 8.8. Bacterial blight disease reaction of Polpully isolate (XPLY-5) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 2 | Yellow lesion | 35.91[b] | 81.76[b] | 29.36[c] | 10.46[c] | Yellowing, whitening and drying |
| IRBB -5 | 2 | Yellow lesion | 36.09[b] | 83.58[c] | 30.16[c] | 10.72[c] | Yellowing, whitening and drying |
| IRBB -13 | 2 | Yellow lesion | 37.98[c] | 86.17[e] | 32.72[d] | 11.38[d] | Yellowing, whitening and drying |
| IRBB -21 | 2 | Yellow lesion | 36.93[b] | 85.69[e] | 31.64[c] | 11.10[c] | Yellowing, whitening and drying |
| IRBB -57 | 2 | Yellow lesion | 30.51[b] | 74.91[a] | 22.85[b] | 10.20[b] | Yellowing, whitening and drying |
| IRBB- 60 | 2 | Yellow lesion | 20.24[a] | 40.14[a] | 8.12[a] | 4.14[a] | Yellowing, whitening and drying |
| Ajaya | 2 | Yellow lesion | 45.92[e] | 85.29[d] | 39.16[e] | 15.20[e] | Yellowing, whitening and drying |
| IR-8 | 2 | Yellow lesion | 46.01[e] | 85.08[d] | 39.06[e] | 15.10[e] | Yellowing, whitening and drying |
| IR-24 | 2 | Yellow lesion | 45.92[e] | 85.39[d] | 39.21[e] | 15.26[e] | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

were noticed on second day after inoculation on six near isogenic lines and three rice differentials.

**4.5.2.9. Akamala isolate** (XAKA-2)

As presented in Table 8.9, the near isogenic line IRBB-60 showed the CI value of 8.27 and lesion length of 4.54 cm found resistant to the isolate. The other near isogenic lines showed CI value ranged from 9.07 to 9.57, lesion length ranged from 5.18 cm to 7.12 cm found moderately resistant to the isolate.The three rice differentials showed the CI value ranged from 20.94 to 39.73 and found to be moderately susceptible to the isolate and showed lesion length ranged from 10.93 cm to 12.44 cm. It was found that the initial symptom of yellow lesion was noticed on third day after inoculation on six near isogenic lines and on three rice differentials.

**4.5.2.10. Kodakara isolate** (XKDA-1)

The Kodakara isolate showed the CI value of 8.10, and the lesion length of 4.58 cm on IRBB-60 found resistant to the pathogen (Table 8.10). It showed CI values ranged from 16.11 to 18.32 and lesion length ranged from 7.12 cm to 8.28 cm, on other five near isogenic lines found moderately resistant.The three rice differentials showed the CI values ranged from 27.13 to 31.06 and lesion length ranged from 9.96 cm to 11.08 cm found moderately susceptible. The isolate took three days to show the initial symptom of yellow lesion on six near isogenic lines and on three rice differentials.

**4.5.2.11. Mannuthy isolate** (XMTY-2)

The near isogenic line IRBB-60 showed the CI value of 8.26 and lesion length of 4.90 cm found resistant to the Mannuthy isolate (Table 8.11).The other near isogenic lines showed the C.I values ranged from 10.80 to 16.10 and lesion length ranged from 5.60 to 7.02 cm found moderately resistant to the pathogen. The three rice differentials showed the CI values ranged from 19.20 to 41.71 and lesion length ranged from 10.10 cm to 13.90 cm found moderately susceptible to the isolate. The initial symptom of yellow lesion was noticed on third day after inoculation on six near isogenic lines and on three rice differentials.

**Table 8.9. Bacterial blight disease reaction of Akamala isolate (XAKA-2 ) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 18.00[a] | 50.68[a] | 9.12[a] | 5.28[a] | Yellowing, whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 19.01[a] | 50.38[a] | 9.57[a] | 7.12[a] | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 18.18[a] | 52.07[a] | 9.46[a] | 5.82[a] | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 18.72[a] | 51.06[a] | 9.55[a] | 6.12[a] | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 19.00[a] | 47.75[a] | 9.07[a] | 5.18[a] | Yellowing, whitening and drying |
| IRBB - 60 | 3 | Yellow lesion | 16.73[a] | 49.47[a] | 8.27[a] | 4.54[a] | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 30.69[b] | 68.26[b] | 20.94[b] | 10.93[b] | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 46.70[c] | 84.47[c] | 39.44[c] | 11.98[c] | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 47.25[c] | 84.10[c] | 39.73[c] | 12.44[c] | Yellowing, whitening and drying |

**Table 8.10. Bacterial blight disease reaction of Kodakara isolate (XKDA-1) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 25.09[b] | 64.21[b] | 16.11[b] | 7.12[b] | Yellowing, whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 24.67[c] | 68.61[b] | 16.92[b] | 7.24[b] | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 24.91[c] | 68.37[b] | 17.03[b] | 7.66[b] | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 26.33[c] | 69.59[b] | 18.32[b] | 8.28[c] | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 20.67[c] | 62.61[b] | 12.20[b] | 5.38[a] | Yellowing, whitening and drying |
| IRBB- 60 | 3 | Yellow lesion | 15.71[a] | 53.57[a] | 8.41[a] | 4.58[a] | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 39.73[d] | 79.54[d] | 31.60[c] | 11.08[d] | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 45.29[e] | 86.88[c] | 39.34[d] | 14.96[e] | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 46.00[e] | 85.85[e] | 39.49[d] | 14.98[e] | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

**Table 8.11. Bacterial blight disease reaction of Mannuthy isolate (XMTY-2) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 24.06$^{c}$ | 66.95$^{c}$ | 16.10$^{c}$ | 7.02$^{c}$ | Yellowing,whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 23.60$^{c}$ | 65.05$^{c}$ | 15.35$^{c}$ | 6.78$^{c}$ | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 23.27$^{c}$ | 60.34$^{b}$ | 14.04$^{c}$ | 6.54$^{c}$ | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 23.01$^{c}$ | 60.72$^{b}$ | 13.97$^{c}$ | 6.46$^{c}$ | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 18.90$^{b}$ | 57.21$^{b}$ | 10.80$^{b}$ | 5.60$^{b}$ | Yellowing, whitening and drying |
| IRBB- 60 | 3 | Yellow lesion | 15.83$^{a}$ | 52.19$^{a}$ | 8.26$^{a}$ | 4.90$^{a}$ | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 27.69$^{d}$ | 69.37$^{c}$ | 19.20$^{d}$ | 10.10$^{d}$ | Yellowing, whitening and drying |
| IR- 8 | 3 | Yellow lesion | 49.14$^{e}$ | 84.49$^{d}$ | 41.51$^{e}$ | 13.26$^{e}$ | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 49.23$^{e}$ | 84.74$^{d}$ | 41.71$^{e}$ | 13.90$^{e}$ | Yellowing, whitening and drying |

**Table 8.12. Bacterial blight disease reaction of Edathua isolate (XEDA-3) on near isogenic lines and rice differentials**

| Variety | *Incubation period(days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 25.09$^{b}$ | 64.21$^{b}$ | 16.10$^{b}$ | 7.08$^{b}$ | Yellowing,whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 22.35$^{b}$ | 61.24$^{a}$ | 13.68$^{b}$ | 6.34$^{b}$ | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 20.00$^{a}$ | 51.77$^{a}$ | 10.35$^{b}$ | 5.58$^{a}$ | Yellowing, whitening and drying |
| IRBB -21 | 3 | Yellow lesion | 23.12$^{b}$ | 62.24$^{a}$ | 14.38$^{b}$ | 6.48$^{b}$ | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 20.84$^{b}$ | 49.54$^{a}$ | 10.32$^{a}$ | 5.10$^{a}$ | Yellowing, whitening and drying |
| IRBB- 60 | 3 | Yellow lesion | 14.71$^{a}$ | 48.78$^{a}$ | 7.17$^{a}$ | 4.14$^{a}$ | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 32.96$^{c}$ | 71.03$^{c}$ | 23.41$^{c}$ | 10.01$^{c}$ | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 44.01$^{c}$ | 89.28$^{d}$ | 39.29$^{d}$ | 14.23$^{d}$ | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 46.06$^{c}$ | 89.49$^{d}$ | 41.21$^{d}$ | 14.58$^{d}$ | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

**4.5.2.12. Edathua isolate** (XEDA-3)

As the results presented in Table 8.12, the isolate showed the CI value of 7.17 and lesion length of 4.14 cm on IRBB-60 found resistant. It showed CI values ranged from 10.32 to 16.10 and lesion length ranged from 5.10 to 7.08 cm to other near isogenic lines, found moderately resistant to the isolate. The three rice differentials showed the CI value ranged from 23.41 to 41.21 and lesion length ranged from 10.01 cm to 14.58 cm, found moderately susceptible to the isolate. The Edathua isolate took three days to show the initial symptom of yellow lesion on six near isogenic lines and on three rice differentials.

**4.5.2.13. Karuvatta isolate** (XKVA-1)

As per the results presented in Table 8.13, IRBB-60 showed the CI value of 8.82 and lesion length of 5.00 cm, found resistant to the isolate. The other near isogenic lines showed the CI values ranged from 9.40 to 18.32 and lesion length ranged from 5.10 to 7.66 cm, found moderately resistant to the pathogen. The three rice differentials showed the CI value ranged from 24.60 to 43.12 and lesion length ranged from 10.03 cm to 14.22 cm, found moderately susceptible to the isolate. It was found that the initial symptom of yellow lesion was noticed on third day after inoculation on all the cases.

**4.5.2.14. Moncombu isolate** (XMOU-1)

The near isogenic lines and rice differentials showed CI values ranged from 0.28 to 3.84 and lesion length ranged from 0.24 cm to 2.84 cm, found resistant to the pathogen (Table 8.14).Yellow lesion as initial symptom appeared on the cut ends on seventh day after inoculation on all the cases.

The cumulative results of the pathogenic variability of 14 isolates on six near isogenic lines and rice differentials are presented in the Table 9a. The study showed that the five isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5)showed moderately susceptible reaction on IRBB-4(*Xa4*), IRBB5(*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) and IRBB-57 (*Xa4/xa5/Xa21*) and susceptible reaction on

**Table 8.13. Bacterial blight disease reaction of Karuvatta isolate (XKVA-1) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 3 | Yellow lesion | 22.67[a] | 63.41[a] | 14.37[b] | 6.50[a] | Yellowing,whitening and drying |
| IRBB -5 | 3 | Yellow lesion | 26.33[c] | 69.59[b] | 18.32[d] | 7.66[c] | Yellowing, whitening and drying |
| IRBB -13 | 3 | Yellow lesion | 19.99[a] | 54.60[a] | 10.91[b] | 5.84[a] | Yellowing, whitening and drying |
| IRBB-21 | 3 | Yellow lesion | 24.67[b] | 66.27[a] | 16.34[c] | 7.02[b] | Yellowing, whitening and drying |
| IRBB -57 | 3 | Yellow lesion | 18.36[a] | 51.25[a] | 9.40[a] | 5.10[a] | Yellowing, whitening and drying |
| IRBB - 60 | 3 | Yellow lesion | 17.39[a] | 50.74[a] | 8.82[a] | 5.00[a] | Yellowing, whitening and drying |
| Ajaya | 3 | Yellow lesion | 33.53[d] | 73.38[b] | 24.60[e] | 10.03[d] | Yellowing, whitening and drying |
| IR-8 | 3 | Yellow lesion | 47.86[d] | 89.30[c] | 42.73[f] | 14.00[e] | Yellowing, whitening and drying |
| IR-24 | 3 | Yellow lesion | 48.26[e] | 89.36[c] | 43.12[f] | 14.22[e] | Yellowing, whitening and drying |

**Table 8.14. Bacterial blight disease reaction of Moncombu isolate (XMOU-1) on near isogenic lines and rice differentials**

| Variety | *Incubation period (days) | Initial symptom expression | PDI | PDS | CI | Lesion length(cm) | Symptom expression |
|---|---|---|---|---|---|---|---|
| IRBB- 4 | 7 | Yellow lesion | 7.93[c] | 30.51[c] | 2.41[c] | 1.42[b] | Yellowing,whitening and drying |
| IRBB -5 | 7 | Yellow lesion | 7.22[c] | 31.16[c] | 2.24[b] | 1.36[b] | Yellowing, whitening and drying |
| IRBB -13 | 7 | Yellow lesion | 5.89[b] | 28.15[b] | 1.65[b] | 0.92[a] | Yellowing, whitening and drying |
| IRBB -21 | 7 | Yellow lesion | 7.93[c] | 30.89[c] | 2.44[c] | 1.48[b] | Yellowing, whitening and drying |
| IRBB -57 | 7 | Yellow lesion | 4.75[b] | 21.36[b] | 1.01[a] | 0.72[a] | Yellowing, whitening and drying |
| IRBB - 60 | 7 | Yellow lesion | 2.27[a] | 12.65[a] | 0.28[a] | 0.24[a] | Yellowing, whitening and drying |
| Ajaya | 7 | Yellow lesion | 9.51[d] | 36.92[c] | 3.51[e] | 2.54[c] | Yellowing, whitening and drying |
| IR-8 | 7 | Yellow lesion | 9.59[d] | 40.06[c] | 3.84[e] | 2.84[c] | Yellowing, whitening and drying |
| IR-24 | 7 | Yellow lesion | 9.81[d] | 35.55[c] | 3.48[d] | 2.42[c] | Yellowing, whitening and drying |

* Incubation period refers to the time taken (days) for expression of initial symptom
Values under same subscript form a homogenous sub group

**Table 9a. Pathotype study of *Xoo* isolates on near isogenic lines and rice differentials**

| NIL/RD / Isolates | IRBB4 | IRBB5 | IRBB13 | IRBB 21 | IRBB 57 | IRBB 60 | Ajaya | IR 8 | IR 24 |
|---|---|---|---|---|---|---|---|---|---|
| XAMI-3 | MS | MS | MS | MS | MS | R | S | S | S |
| XERM-1 | MS | MS | MS | MS | MS | R | S | S | S |
| XKOR-3 | R | R | R | R | R | R | R | R | R |
| XMRA-2 | MR | MR | MR | MR | MR | R | MS | S | S |
| XNRA-1 | MS | MS | MS | MS | MS | R | S | S | S |
| XPAI-3 | MS | MS | MS | MS | MS | R | S | S | S |
| XPTB-4 | MS | MR | MS | MR | MR | R | MR | S | S |
| XPLY-5 | MS | MS | MS | MS | MS | R | S | S | S |
| XAKA-2 | MR | MR | MR | MR | MR | R | MS | S | S |
| XKDA-1 | MR | MR | MR | MR | MR | R | MS | S | S |
| XMTY-2 | MR | MR | MR | MR | MR | R | MR | S | S |
| XEDA-3 | MR | MR | MR | MR | MR | R | MS | S | S |
| XKVA-1 | MR | MR | MR | MR | MR | R | MS | S | S |
| XMOU-1 | R | R | R | R | R | R | R | R | R |

R- Resistant MR- Moderately resistant MS-Moderately susceptible S- Susceptible

the three rice differentials (Ajaya, IR-8 and IR-24).The seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) showed moderately resistant reaction on IRBB-4 (*Xa4*),IRBB-5(*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) and IRBB-57 (*Xa4/xa5/Xa21*). Moderately resistant to moderately susceptible reaction was observed on Ajaya and susceptible reaction on IR-8 and IR-24. The two isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed resistant reaction on all the five near isogenic lines and on rice differentials. All the fourteen isolates showed resistant reaction to the near isogenic line IRBB-60.

Reaction of near isogenic line IRBB-60 to 14 isolates of *Xoo* is presented in Table 9b. The 14 isolates showed the PDI, PDS and CI values ranged from 2.27 – 26.56, 12.65 – 69.02 and 0.28 – 8.82 respectively on the near isogenic line IRBB-60. They showed the lesion length ranged from 0.24 – 4.90 cm. The isolates showed the initial symptom of yellow lesion at 2 to 7 days interval. Based on these observations, it can be stated that the near isogenic line IRBB-60 is identified as resistant to all the fourteen isolates of *Xoo*.

### 4.5.3. Studies on kresek reaction of *Xoo* isolates on selected rice varieties

To study the kresek symptom inciting ability of 14 isolates of *Xoo* obtained from three districts of Kerala *viz.*, Alappuzha, Palakkad and Thrissur were studied by treating 20 cultivated rice varieties as mentioned under materials and methods. The results of the study are presented below:

#### 4.5.3.1. Athimani isolate (XAMI-3)

From the data, it was found that the varieties showed the CI values ranged from 39.51 to 48.97 and found susceptible to the pathogen (Table 10.1). All the twenty varieties showed the initial symptom of yellowing with water soaked brown lesion on fifth day after sowing the pathogen treated seeds. It was followed by inward rolling and wilting.

**Table 9b. Bacterial blight disease reaction of near isogenic line IRBB-60 to fourteen isolates of *Xoo***

| Sl.No. | Isolates | Incubation period (days) | Initial symptom expression | 21 DAI | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | PDI | PDS | CI | Disease reaction | Lesion length (cm) | Disease reaction |
| 1 | Athimani (XAMI-3) | 2 | Yellow lesion | 15.78 | 50.38 | 7.94 | R | 4.54 | R |
| 2 | Erattakulam (XERM-1) | 2 | Yellow lesion | 26.56 | 69.02 | 4.52 | R | 4.70 | R |
| 3 | Kodallur (XKOR-3) | 7 | Yellow lesion | 3.23 | 18.20 | 0.58 | R | 0.50 | R |
| 4 | Manchira (XMRA-2) | 3 | Yellow lesion | 11.25 | 41.92 | 4.71 | R | 3.42 | R |
| 5 | Nenmara (XNRA-1) | 2 | Yellow lesion | 20.57 | 40.71 | 8.37 | R | 4.82 | R |
| 6 | Parali (XPAI-3) | 2 | Yellow lesion | 20.94 | 40.37 | 8.45 | R | 4.60 | R |
| 7 | Pattambi (XPTB-4 ) | 3 | Yellow lesion | 16.07 | 49.87 | 8.27 | R | 4.44 | R |
| 8 | Polpully (XPLY-5) | 2 | Yellow lesion | 20.24 | 40.14 | 8.12 | R | 4.14 | R |
| 9 | Akamala (XAKA-2) | 3 | Yellow lesion | 16.73 | 49.47 | 8.27 | R | 4.54 | R |
| 10 | Kodakara (XKDA-1) | 3 | Yellow lesion | 15.71 | 53.57 | 8.41 | R | 4.58 | R |
| 11 | Mannuthy (XMTY-2) | 3 | Yellow lesion | 15.83 | 52.19 | 8.26 | R | 4.90 | R |
| 12 | Edathua (XEDA-3) | 3 | Yellow lesion | 14.71 | 48.78 | 7.17 | R | 4.14 | R |
| 13 | Karuvatta (XKVA-1) | 3 | Yellow lesion | 17.39 | 50.74 | 8.82 | R | 5.00 | R |
| 14 | Moncombu (XMOU-1) | 7 | Yellow lesion | 2.27 | 12.65 | 0.28 | R | 0.24 | R |

R- Resistant

**4.5.3.2. Erattakulam isolate** (XERM-1)

As presented in Table 10.2, the varieties showed the CI values ranged from 40.93 to 53.72 due to the pathogen infection and grouped the varieties as susceptible. Twenty varieties were showed the initial symptom of yellowing with water soaked brown lesion on fifth day after sowing, followed by inward rolling and wilting.

**4.5.3.3. Kodallur isolate** (XKOR-3)

As seen in the Table 10.3, the isolate showed the CI values ranged from 1.95 to 6.32 and grouped as resistant. All the twenty varieties were showed the initial symptom of yellowing with water soaked brown lesion on fifteen days after sowing the pathogen treated seeds. It was followed by inward rolling and wilting.

**4.5.3.4. Manchira isolate** (XMRA-2)

The kresek symptom inciting ability of the Manchira isolate is presented in the Table 10.4. It was found that the varieties showed the CI values ranged from 29.83 to 38.83 and grouped as moderately susceptible. The initial symptom of yellowing with water soaked brown lesion on all the varieties were observed on seven days after sowing and in the advanced stage the pathogen produced inward rolling and wilting of the affected plants.

**4.5.3.5. Nenmara isolate** (XNRA-1)

The results of the Nenmara isolate to initiate the kresek symptom on twenty varieties is presented in the Table 10.5.The pathogen showed the CI values ranged from 40.14 to 46.09 on all twenty varieties used in the study and grouped as susceptible. All the varieties showed the initial symptom of yellowing with water soaked brown lesion on five days after sowing, followed by inward rolling and wilting.

**4.5.3.6. Parali isolate** (XPAI-3)

As seen in the Table 10.6, it was found that the varieties showed the CI values ranged from 40.79 to 49.71 and grouped as susceptible. All the varieties were showed yellowing with water soaked brown lesion on five days after sowing the pathogen treated seeds and followed by inward rolling and wilting in the advanced stage of infection.

**4.5.3.7. Pattambi isolate** (XPTB-4)

As presented in the Table 10.7, it was found that all the varieties showed the CI values ranged from 32.07 to 38.17 and grouped as moderately susceptible. All the varieties showed the initial symptom of yellowing with water soaked brown lesion on seven days after sowing of the treated seeds and in the later stage, it produced inward rolling and wilting of the plants.

**4.5.3.8. Polpully isolate** (XPLY-5)

Regarding the Polpully isolate, it showed the CI values ranged from 40.14 to 45.19 on 20 varieties and the varieties were grouped as susceptible (Table 10.8). All the twenty varieties showed the initial symptom of yellowing with water soaked brown lesion on five days after sowing the pathogen treated seeds, followed by inward rolling and wilting of the plants.

**4.5.3.9. Akamala isolate** (XAKA-2)

The Akamala isolate, on infection showed the CI values ranged from 34.02 to 38.99 and grouped all the varieties as moderately susceptible (Table 10.9).Twenty varieties showed the initial symptom of yellowing with water soaked brown lesion on seven days after sowing the pathogen treated seeds. It was followed by inward rolling and wilting.

**4.5.3.10. Kodakara isolate** (XKDA-1)

The Kodakara isolate on infection showed the CI values ranged from 24.18 to 37.92 on 20 varieties and grouped them as moderately susceptible (Table 10.10). Initial symptom of yellowing with water soaked brown lesion was observed on seven days after sowing the pathogen treated seeds. In the later stage of infection the pathogen produced inward rolling and wilting of the plants.

**4.5.3.11. Mannuthy isolate** (XMTY-2)

Infection of the Mannuthy isolate on 20 varieties showed the CI values ranged from 32.24 to 37.35 and grouped them as moderately susceptible (Table 10.11).Yellowing with water soaked brown lesion, on seven days after sowing the pathogen treated seeds on all the varieties was observed and was followed by inward rolling and wilting.

**4.5.3.12. Edathua isolate** (XEDA-3)

As seen in the Table 10.12, it was found that the varieties showed the CI values ranged from 24.35 to 29.72 due the infection of the isolate and the varieties were grouped as moderately susceptible. All the varieties were showed the initial symptom of yellowing with water soaked brown lesion on seven days after sowing the pathogen treated seeds and followed by inward rolling and wilting was observed.

**4.5.3.13. Karuvatta isolate** (XKVA-1)

The Karuvatta isolate showed the initial symptom of yellowing with water soaked brown lesion on all the varieties on seven days after sowing. In the later stage it could produce inward rolling and wilting of the plants. It was found that the varieties showed the CI values ranged from 19.25 to 28.56 and them grouped as moderately susceptible (Table 10.13).

**Table 10.1.Kresek symptom development of Athimani isolate (XAMI-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 5 | 55.76$^{e}$ | 83.80$^{a}$ | 46.72$^{d}$ | Yellowing and wilting |
| 2 | Anashwara | 5 | 55.33$^{e}$ | 87.30$^{b}$ | 48.30$^{e}$ | Yellowing and wilting |
| 3 | Annapoorna | 5 | 53.04$^{c}$ | 83.14$^{a}$ | 44.09$^{c}$ | Yellowing and wilting |
| 4 | Aswathi | 5 | 54.96$^{c}$ | 81.33$^{a}$ | 44.69$^{c}$ | Yellowing and wilting |
| 5 | Bhadra | 5 | 57.30$^{f}$ | 85.23$^{a}$ | 48.83$^{f}$ | Yellowing and wilting |
| 6 | Bharathy | 5 | 54.72$^{e}$ | 89.50$^{b}$ | 48.97$^{f}$ | Yellowing and wilting |
| 7 | Harsha | 5 | 54.47$^{d}$ | 83.61$^{a}$ | 45.54$^{c}$ | Yellowing and wilting |
| 8 | Jaya | 5 | 56.74$^{f}$ | 82.66$^{a}$ | 46.90$^{e}$ | Yellowing and wilting |
| 9 | Jyothi | 5 | 56.11$^{e}$ | 81.66$^{a}$ | 45.81$^{c}$ | Yellowing and wilting |
| 10 | Kairali | 5 | 55.85$^{e}$ | 85.11$^{a}$ | 47.53$^{e}$ | Yellowing and wilting |
| 11 | Kanchana | 5 | 53.77$^{d}$ | 82.66$^{a}$ | 44.44$^{c}$ | Yellowing and wilting |
| 12 | Karuna | 5 | 53.77$^{d}$ | 82.66$^{a}$ | 44.44$^{c}$ | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 5 | 48.85$^{a}$ | 80.99$^{a}$ | 39.56$^{a}$ | Yellowing and wilting |
| 14 | Manupriya | 5 | 50.24$^{a}$ | 83.61$^{a}$ | 42.00$^{b}$ | Yellowing and wilting |
| 15 | Matta Triveni | 5 | 53.04$^{c}$ | 83.14$^{a}$ | 44.09$^{c}$ | Yellowing and wilting |
| 16 | Neeraja | 5 | 54.47$^{d}$ | 83.61$^{a}$ | 45.54$^{c}$ | Yellowing and wilting |
| 17 | Rohini | 5 | 48.59$^{a}$ | 81.33$^{a}$ | 39.51$^{a}$ | Yellowing and wilting |
| 18 | Sabari | 5 | 54.72$^{e}$ | 89.50$^{b}$ | 48.97$^{f}$ | Yellowing and wilting |
| 19 | Swetha | 5 | 51.61$^{b}$ | 82.61$^{a}$ | 42.63$^{b}$ | Yellowing and wilting |
| 20 | Uma | 5 | 55.85$^{e}$ | 83.61$^{a}$ | 46.69$^{d}$ | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.2.Kresek symptom development of Erattakulam isolate (XERM-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 5 | $60.31^{f}$ | $85.95^{a}$ | $51.83^{g}$ | Yellowing and wilting |
| 2 | Anashwara | 5 | $59.60^{e}$ | $86.42^{a}$ | $51.50^{g}$ | Yellowing and wilting |
| 3 | Annapoorna | 5 | $60.40^{f}$ | $84.33^{a}$ | $50.93^{f}$ | Yellowing and wilting |
| 4 | Aswathi | 5 | $57.79^{d}$ | $85.59^{a}$ | $49.46^{e}$ | Yellowing and wilting |
| 5 | Bhadra | 5 | $57.14^{c}$ | $83.42^{a}$ | $47.66^{d}$ | Yellowing and wilting |
| 6 | Bharathy | 5 | $55.55^{b}$ | $83.97^{a}$ | $46.64^{c}$ | Yellowing and wilting |
| 7 | Harsha | 5 | $53.01^{a}$ | $83.49^{a}$ | $44.25^{a}$ | Yellowing and wilting |
| 8 | Jaya | 5 | $49.54^{a}$ | $82.64^{a}$ | $40.93^{a}$ | Yellowing and wilting |
| 9 | Jyothi | 5 | $53.65^{a}$ | $84.75^{a}$ | $45.46^{b}$ | Yellowing and wilting |
| 10 | Kairali | 5 | $51.16^{a}$ | $85.23^{a}$ | $43.60^{a}$ | Yellowing and wilting |
| 11 | Kanchana | 5 | $51.74^{a}$ | $84.75^{a}$ | $43.84^{a}$ | Yellowing and wilting |
| 12 | Karuna | 5 | $52.00^{a}$ | $85.23^{a}$ | $44.31^{a}$ | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 5 | $51.03^{a}$ | $86.42^{a}$ | $44.10^{a}$ | Yellowing and wilting |
| 14 | Manupriya | 5 | $50.41^{a}$ | $84.56^{a}$ | $42.62^{a}$ | Yellowing and wilting |
| 15 | Matta Triveni | 5 | $50.25^{a}$ | $84.09^{a}$ | $42.25^{a}$ | Yellowing and wilting |
| 16 | Neeraja | 5 | $50.58^{a}$ | $84.75^{a}$ | $42.86^{a}$ | Yellowing and wilting |
| 17 | Rohini | 5 | $53.86^{a}$ | $83.80^{a}$ | $45.13^{b}$ | Yellowing and wilting |
| 18 | Sabari | 5 | $52.15^{a}$ | $84.45^{a}$ | $44.04^{a}$ | Yellowing and wilting |
| 19 | Swetha | 5 | $55.71^{b}$ | $83.14^{a}$ | $46.31^{b}$ | Yellowing and wilting |
| 20 | Uma | 5 | $62.38^{g}$ | $86.12^{b}$ | $53.72^{h}$ | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.3.Kresek symptom development of Kodallur isolate (XKOR-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 15 | $7.67^{a}$ | $53.33^{b}$ | $4.09^{a}$ | Yellowing and wilting |
| 2 | Anashwara | 15 | $7.78^{a}$ | $41.10^{a}$ | $3.19^{a}$ | Yellowing and wilting |
| 3 | Annapoorna | 15 | $5.63^{a}$ | $44.00^{a}$ | $2.47^{a}$ | Yellowing and wilting |
| 4 | Aswathi | 15 | $8.20^{a}$ | $45.43^{a}$ | $3.73^{a}$ | Yellowing and wilting |
| 5 | Bhadra | 15 | $7.37^{a}$ | $39.67^{a}$ | $2.92^{a}$ | Yellowing and wilting |
| 6 | Bharathy | 15 | $7.15^{a}$ | $43.00^{a}$ | $3.07^{a}$ | Yellowing and wilting |
| 7 | Harsha | 15 | $7.67^{a}$ | $53.33^{b}$ | $4.09^{a}$ | Yellowing and wilting |
| 8 | Jaya | 15 | $8.01^{a}$ | $51.71^{b}$ | $4.14^{a}$ | Yellowing and wilting |
| 9 | Jyothi | 15 | $7.52^{a}$ | $39.67^{a}$ | $2.98^{a}$ | Yellowing and wilting |
| 10 | Kairali | 15 | $7.63^{a}$ | $62.00^{c}$ | $4.73^{b}$ | Yellowing and wilting |
| 11 | Kanchana | 15 | $11.85^{d}$ | $53.33^{b}$ | $6.32^{d}$ | Yellowing and wilting |
| 12 | Karuna | 15 | $7.78^{a}$ | $41.10^{a}$ | $3.19^{a}$ | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 15 | $8.30^{a}$ | $37.33^{a}$ | $3.09^{a}$ | Yellowing and wilting |
| 14 | Manupriya | 15 | $4.66^{a}$ | $42.00^{a}$ | $1.95^{a}$ | Yellowing and wilting |
| 15 | Matta Triveni | 15 | $8,07^{a}$ | $44.67^{a}$ | $3.60^{a}$ | Yellowing and wilting |
| 16 | Neeraja | 15 | $8.26^{a}$ | $39.67^{a}$ | $3.27^{a}$ | Yellowing and wilting |
| 17 | Rohini | 15 | $7.36^{a}$ | $46.86^{a}$ | $3.44^{a}$ | Yellowing and wilting |
| 18 | Sabari | 15 | $9.46^{c}$ | $51.43^{b}$ | $4.86^{b}$ | Yellowing and wilting |
| 19 | Swetha | 15 | $6.78^{a}$ | $35.00^{a}$ | $2.37^{a}$ | Yellowing and wilting |
| 20 | Uma | 15 | $8.86^{b}$ | $70.24^{d}$ | $6.22^{c}$ | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.4.Kresek symptom development of Manchira isolate (XMRA-2) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period (days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 42.85[a] | 80.32[a] | 34.41[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 46.82[a] | 78.67[a] | 36.83[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 42.63[a] | 80.33[a] | 34.24[a] | Yellowing and wilting |
| 4 | Aswathi | 7 | 45.74[a] | 80.33[a] | 36.74[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 43.89[a] | 79.00[a] | 34.67[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 47.56[a] | 81.66[b] | 38.83[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 44.30[a] | 81.33[a] | 36.02[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 46.22[a] | 78.00[a] | 36.05[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 39.26[a] | 76.00[a] | 29.83[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 39.81[a] | 77.76[a] | 30.95[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 43.89[a] | 79.00[a] | 34.67[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 46.11[a] | 80.11[a] | 36.93[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 42.78[a] | 79.00[a] | 33.79[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 44.67[a] | 80.67[a] | 36.03[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 45.52[a] | 79.67[a] | 36.26[a] | Yellowing and wilting |
| 16 | Neeraja | 7 | 42.85[a] | 80.32[a] | 34.41[a] | Yellowing and wilting |
| 17 | Rohini | 7 | 45.86[a] | 79.33[a] | 36.38[a] | Yellowing and wilting |
| 18 | Sabari | 7 | 48.05[a] | 76.40[a] | 36.53[a] | Yellowing and wilting |
| 19 | Swetha | 7 | 39.81[a] | 77.76[a] | 30.95[a] | Yellowing and wilting |
| 20 | Uma | 7 | 46.22[a] | 78.00[a] | 36.05[a] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.5.Kresek symptom development of Nenmara isolate (XNRA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 5 | 50.82[a] | 79.67[a] | 40.48[a] | Yellowing and wilting |
| 2 | Anashwara | 5 | 55.61[b] | 81.81[a] | 45.49[d] | Yellowing and wilting |
| 3 | Annapoorna | 5 | 51.27[a] | 81.81[a] | 41.94[a] | Yellowing and wilting |
| 4 | Aswathi | 5 | 51.70[a] | 81.33[a] | 42.04[a] | Yellowing and wilting |
| 5 | Bhadra | 5 | 55.72[b] | 80.81[a] | 45.02[c] | Yellowing and wilting |
| 6 | Bharathy | 5 | 51.78[a] | 81.33[a] | 42.11[a] | Yellowing and wilting |
| 7 | Harsha | 5 | 50.15[a] | 80.67[a] | 40.45[a] | Yellowing and wilting |
| 8 | Jaya | 5 | 48.85[a] | 80.99[a] | 39.56[a] | Yellowing and wilting |
| 9 | Jyothi | 5 | 50.15[a] | 80.67[a] | 40.45[a] | Yellowing and wilting |
| 10 | Kairali | 5 | 48.96[a] | 81.99[a] | 40.14[a] | Yellowing and wilting |
| 11 | Kanchana | 5 | 56.35[c] | 81.81[a] | 46.09[c] | Yellowing and wilting |
| 12 | Karuna | 5 | 51.70[a] | 81.33[a] | 42.04[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 5 | 50.75[a] | 82.47[a] | 41.85[a] | Yellowing and wilting |
| 14 | Manupriya | 5 | 49.40[a] | 82.28[a] | 40.64[a] | Yellowing and wilting |
| 15 | Matta Triveni | 5 | 50.67[a] | 82.66[a] | 41.88[a] | Yellowing and wilting |
| 16 | Neeraja | 5 | 53.20[a] | 84.09[b] | 44.73[b] | Yellowing and wilting |
| 17 | Rohini | 5 | 55.72[b] | 80.81[a] | 45.02[c] | Yellowing and wilting |
| 18 | Sabari | 5 | 52.18[a] | 80.99[a] | 42.26[a] | Yellowing and wilting |
| 19 | Swetha | 5 | 51.27[a] | 81.81[a] | 41.94[a] | Yellowing and wilting |
| 20 | Uma | 5 | 52.06[a] | 85.71[c] | 44.62[b] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

Table 10.6.Kresek symptom development of Parali isolate (XPAI-3) on twenty cultivated rice varieties

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 5 | 53.89[a] | 81.66[a] | 44.00[a] | Yellowing and wilting |
| 2 | Anashwara | 5 | 59.80[c] | 83.14[a] | 49.71[e] | Yellowing and wilting |
| 3 | Annapoorna | 5 | 54.26[a] | 80.99[a] | 43.94[a] | Yellowing and wilting |
| 4 | Aswathi | 5 | 54.45[a] | 83.97[a] | 45.72[c] | Yellowing and wilting |
| 5 | Bhadra | 5 | 57.70[c] | 82.62[a] | 47.67[d] | Yellowing and wilting |
| 6 | Bharathy | 5 | 55.37[a] | 81.66[a] | 45.21[b] | Yellowing and wilting |
| 7 | Harsha | 5 | 53.07[a] | 80.33[a] | 42.63[a] | Yellowing and wilting |
| 8 | Jaya | 5 | 57.02[b] | 82.47[a] | 47.02[d] | Yellowing and wilting |
| 9 | Jyothi | 5 | 53.63[b] | 81.33[a] | 43.61[a] | Yellowing and wilting |
| 10 | Kairali | 5 | 57.63[c] | 81.33[a] | 46.87[d] | Yellowing and wilting |
| 11 | Kanchana | 5 | 56.53[a] | 80.81[a] | 45.68[c] | Yellowing and wilting |
| 12 | Karuna | 5 | 56.03[b] | 84.28[a] | 47.22[d] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 5 | 50.30[a] | 81.10[a] | 40.79[a] | Yellowing and wilting |
| 14 | Manupriya | 5 | 50.30[a] | 81.10[a] | 40.79[a] | Yellowing and wilting |
| 15 | Matta Triveni | 5 | 56.53[b] | 80.81[a] | 45.68[b] | Yellowing and wilting |
| 16 | Neeraja | 5 | 58.11[c] | 82.95[a] | 48.20[e] | Yellowing and wilting |
| 17 | Rohini | 5 | 55.25[a] | 82.31[a] | 45.47[b] | Yellowing and wilting |
| 18 | Sabari | 5 | 54.66[a] | 85.23[b] | 46.58[a] | Yellowing and wilting |
| 19 | Swetha | 5 | 50.26[a] | 80.33[a] | 40.37[a] | Yellowing and wilting |
| 20 | Uma | 5 | 56.89[b] | 82.66[a] | 47.02[d] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.7.Kresek symptom development of Pattambi isolate (XPTB-4) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 45.52[a] | 80.33[a] | 36.56[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 46.63[b] | 80.33[a] | 37.45[b] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 46.07[b] | 81.33[a] | 37.46[b] | Yellowing and wilting |
| 4 | Aswathi | 7 | 44.82[a] | 83.33[b] | 37.34[b] | Yellowing and wilting |
| 5 | Bhadra | 7 | 46.59[b] | 80.67[a] | 37.58[b] | Yellowing and wilting |
| 6 | Bharathy | 7 | 42.48[a] | 79.67[a] | 33.84[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 45.15[a] | 79.67[a] | 35.97[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 45.52[a] | 80.33[a] | 36.56[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 44.44[a] | 80.00[a] | 35.55[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 42.96[a] | 79.33[a] | 34.08[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 45.37[a] | 79.67[a] | 36.14[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 45.74[b] | 80.33[a] | 36.74[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 45.17[a] | 81.33[a] | 36.73[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 45.19[a] | 81.33[a] | 36.75[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 46.63[b] | 80.33[a] | 37.45[b] | Yellowing and wilting |
| 16 | Neeraja | 7 | 47.52[b] | 80.33[a] | 38.17[b] | Yellowing and wilting |
| 17 | Rohini | 7 | 46.48[b] | 81.66[a] | 37.95[b] | Yellowing and wilting |
| 18 | Sabari | 7 | 40.26[a] | 79.67[a] | 32.07[a] | Yellowing and wilting |
| 19 | Swetha | 7 | 46.59[b] | 79.67[a] | 37.11[b] | Yellowing and wilting |
| 20 | Uma | 7 | 48.15[c] | 78.67[a] | 37.87[b] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.8.Kresek symptom development of Polpully isolate (XPLY-5) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | ** 21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 5 | 50.16[a] | 80.95[a] | 40.60[a] | Yellowing and wilting |
| 2 | Anashwara | 5 | 54.67[b] | 82.66[a] | 45.19[d] | Yellowing and wilting |
| 3 | Annapoorna | 5 | 49.63[a] | 81.33[a] | 40.36[a] | Yellowing and wilting |
| 4 | Aswathi | 5 | 52.74[a] | 81.33[a] | 42.89[b] | Yellowing and wilting |
| 5 | Bhadra | 5 | 47.85[a] | 81.33[a] | 38.91[a] | Yellowing and wilting |
| 6 | Bharathy | 5 | 52.49[a] | 81.47[a] | 42.77[b] | Yellowing and wilting |
| 7 | Harsha | 5 | 51.55[a] | 80.00[a] | 41.24[a] | Yellowing and wilting |
| 8 | Jaya | 5 | 50.90[a] | 83.81[b] | 42.65[b] | Yellowing and wilting |
| 9 | Jyothi | 5 | 48.96[a] | 81.99[a] | 40.14[a] | Yellowing and wilting |
| 10 | Kairali | 5 | 51.85[a] | 81.33[a] | 42.16[a] | Yellowing and wilting |
| 11 | Kanchana | 5 | 49.64[a] | 81.81[a] | 40.61[a] | Yellowing and wilting |
| 12 | Karuna | 5 | 50.90[a] | 83.81[b] | 42.71[b] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 5 | 49.54[a] | 82.83[a] | 41.03[a] | Yellowing and wilting |
| 14 | Manupriya | 5 | 50.16[a] | 80.67[a] | 40.46[a] | Yellowing and wilting |
| 15 | Matta Triveni | 5 | 52.37[a] | 80.67[a] | 42.24[a] | Yellowing and wilting |
| 16 | Neeraja | 5 | 49.64[a] | 81.81[a] | 40.61[a] | Yellowing and wilting |
| 17 | Rohini | 5 | 51.85[a] | 81.33[a] | 42.16[a] | Yellowing and wilting |
| 18 | Sabari | 5 | 52.81[a] | 81.99[a] | 43.29[c] | Yellowing and wilting |
| 19 | Swetha | 5 | 49.54[a] | 82.83[a] | 41.03[a] | Yellowing and wilting |
| 20 | Uma | 5 | 50.90[a] | 83.81[b] | 42.65[b] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

Table 10.9.Kresek symptom development of Akamala isolate (XAKA-2) on twenty cultivated rice varieties

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 45.59[a] | 80.99[a] | 36.92[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 45.70[a] | 81.81[a] | 37.38[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 44.70[a] | 80.33[a] | 35.90[a] | Yellowing and wilting |
| 4 | Aswathi | 7 | 47.85[a] | 81.33[a] | 38.91[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 44.30[a] | 82.66[a] | 36.61[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 45.04[a] | 82.66[a] | 37.23[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 48.08[a] | 81.10[a] | 38.99[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 42.89[a] | 79.33[a] | 34.02[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 44.93[a] | 79.67[a] | 35.79[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 44.30[a] | 82.66[a] | 36.61[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 47.15[a] | 79.66[a] | 37.55[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 45.67[a] | 84.33[a] | 38.91[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 45.70[a] | 81.81[a] | 37.38[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 45.59[a] | 80.99[a] | 36.92[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 47.33[a] | 80.67[a] | 38.18[a] | Yellowing and wilting |
| 16 | Neeraja | 7 | 44.30[a] | 82.66[a] | 36.61[a] | Yellowing and wilting |
| 17 | Rohini | 7 | 44.93[a] | 79.67[a] | 35.79[a] | Yellowing and wilting |
| 18 | Sabari | 7 | 44.70[a] | 80.33[a] | 35.90[a] | Yellowing and wilting |
| 19 | Swetha | 7 | 45.28[a] | 81.32[a] | 36.82[a] | Yellowing and wilting |
| 20 | Uma | 7 | 47.34[a] | 81.10[a] | 38.39[a] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

Table 10.10.Kresek symptom development of Kodakara isolate (XKDA-1) on twenty cultivated rice varieties

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 33.67[a] | 77.00[a] | 25.96[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 42.78[d] | 75.00[a] | 32.08[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 45.75[e] | 81.78[a] | 37.92[c] | Yellowing and wilting |
| 4 | Aswathi | 7 | 39.11[a] | 80.00[a] | 31.29[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 38.04[a] | 77.67[a] | 29.65[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 35.56[a] | 78.00[a] | 27.78[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 31.15[a] | 77.00[a] | 24.20[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 41.93[c] | 78.67[a] | 33.02[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 31.56[a] | 78.00[a] | 32.11[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 35.56[a] | 80.00[a] | 31.29[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 40.71[c] | 79.67[a] | 32.45[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 38.78[a] | 77.00[a] | 29.88[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 42.22[c] | 78.00[a] | 32.11[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 38.00[a] | 82.00[b] | 31.53[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 40.30[b] | 77.33[a] | 31.25[a] | Yellowing and wilting |
| 16 | Neeraja | 7 | 31.63[a] | 75.33[a] | 24.18[a] | Yellowing and wilting |
| 17 | Rohini | 7 | 41.63[c] | 81.33[a] | 33.92[b] | Yellowing and wilting |
| 18 | Sabari | 7 | 42.78[d] | 75.00[a] | 32.08[a] | Yellowing and wilting |
| 19 | Swetha | 7 | 35.48[a] | 80.00[a] | 29.06[a] | Yellowing and wilting |
| 20 | Uma | 7 | 38.00[a] | 78.00[a] | 29.79[a] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.11.Kresek symptom development of Mannuthy isolate (XMTY-2) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | ** 21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 41.26[a] | 80.67[a] | 33.28[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 39.85[a] | 81.33[a] | 32.41[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 42.89[a] | 78.00[a] | 33.45[a] | Yellowing and wilting |
| 4 | Aswathi | 7 | 47.48[a] | 78.67[a] | 37.35[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 42.37[a] | 78.67[a] | 33.33[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 45.30[a] | 80.33[a] | 36.38[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 42.67[a] | 81.33[a] | 34.70[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 44.71[a] | 77.67[a] | 34.72[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 41.32[a] | 78.67[a] | 32.50[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 45.30[a] | 80.33[a] | 36.38[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 45.74[a] | 77.67[a] | 35.52[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 43.19[a] | 79.33[a] | 34.26[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 42.82[a] | 78.67[a] | 33.68[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 40.86[a] | 79.81[a] | 32.61[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 42.83[a] | 77.67[a] | 33.26[a] | Yellowing and wilting |
| 16 | Neeraja | 7 | 42.48[a] | 76.33[a] | 32.24[a] | Yellowing and wilting |
| 17 | Rohini | 7 | 44.44[a] | 79.33[a] | 35.25[a] | Yellowing and wilting |
| 18 | Sabari | 7 | 43.93[a] | 78.67[a] | 34.55[a] | Yellowing and wilting |
| 19 | Swetha | 7 | 44.63[a] | 80.10[a] | 35.74[a] | Yellowing and wilting |
| 20 | Uma | 7 | 42.48[a] | 77.87[a] | 33.07[a] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.12.Kresek symptom development of Edathua isolate (XEDA-3) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 7 | 32.19[a] | 75.67[a] | 24.35[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 32.24[a] | 75.98[a] | 24.49[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 32.44[a] | 76.17[a] | 24.70[a] | Yellowing and wilting |
| 4 | Aswathi | 7 | 32.59[a] | 77.17[a] | 25.14[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 32.78[a] | 77.19[a] | 25.30[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 33.19[a] | 77.64[a] | 25.76[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 33.22[a] | 77.77[a] | 25.83[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 33.53[a] | 77.96[a] | 26.13[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 33.68[a] | 78.06[a] | 26.29[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 33.71[a] | 78.16[a] | 26.34[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 34.39[a] | 78.33[a] | 26.92[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 34.56[a] | 78.42[a] | 27.17[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 34.75[a] | 78.66[a] | 27.33[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 34.78[a] | 78.66[a] | 27.35[a] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 35.40[b] | 78.75[a] | 27.87[a] | Yellowing and wilting |
| 16 | Neeraja | 7 | 35.45[b] | 79.11[a] | 28.04[b] | Yellowing and wilting |
| 17 | Rohini | 7 | 35.46[b] | 79.32[a] | 28.12[b] | Yellowing and wilting |
| 18 | Sabari | 7 | 35.78[c] | 79.68[a] | 28.50[b] | Yellowing and wilting |
| 19 | Swetha | 7 | 36.97[d] | 80.40[b] | 29.72[c] | Yellowing and wilting |
| 20 | Uma | 7 | 33.19[a] | 77.64[a] | 25.76[a] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.13.Kresek symptom development of Karuvatta isolate (XKVA-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | C.I | |
| 1 | Aiswarya | 7 | 26.32[a] | 73.15[a] | 19.25[a] | Yellowing and wilting |
| 2 | Anashwara | 7 | 27.16[a] | 73.23[a] | 19.88[a] | Yellowing and wilting |
| 3 | Annapoorna | 7 | 27.72[a] | 73.79[a] | 20.45[a] | Yellowing and wilting |
| 4 | Aswathi | 7 | 27.90[a] | 74.30[a] | 20.72[a] | Yellowing and wilting |
| 5 | Bhadra | 7 | 28.00[a] | 74.92[a] | 20.97[a] | Yellowing and wilting |
| 6 | Bharathy | 7 | 28.67[a] | 74.95[a] | 21.48[a] | Yellowing and wilting |
| 7 | Harsha | 7 | 28.80[a] | 75.21[a] | 21.66[a] | Yellowing and wilting |
| 8 | Jaya | 7 | 29.27[a] | 75.34[a] | 22.05[a] | Yellowing and wilting |
| 9 | Jyothi | 7 | 29.44[a] | 75.96[a] | 22.36[a] | Yellowing and wilting |
| 10 | Kairali | 7 | 29.66[a] | 76.48[a] | 22.68[a] | Yellowing and wilting |
| 11 | Kanchana | 7 | 30.00[a] | 76.58[a] | 22.97[a] | Yellowing and wilting |
| 12 | Karuna | 7 | 30.03[a] | 76.50[a] | 22.97[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 7 | 30.29[a] | 77.26[b] | 23.40[a] | Yellowing and wilting |
| 14 | Manupriya | 7 | 30.71[b] | 77.61[c] | 23.83[b] | Yellowing and wilting |
| 15 | Matta Triveni | 7 | 30.75[b] | 77.81[c] | 23.92[b] | Yellowing and wilting |
| 16 | Neeraja | 7 | 31.71[c] | 78.08[d] | 24.75[c] | Yellowing and wilting |
| 17 | Rohini | 7 | 32.20[d] | 78.67[e] | 25.33[c] | Yellowing and wilting |
| 18 | Sabari | 7 | 33.11[e] | 78.67[e] | 26.04[d] | Yellowing and wilting |
| 19 | Swetha | 7 | 34.30[f] | 79.11[f] | 27.13[e] | Yellowing and wilting |
| 20 | Uma | 7 | 35.97[f] | 79.40[f] | 28.56[f] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

**Table 10.14. Kresek symptom development of Moncombu isolate (XMOU-1) on twenty cultivated rice varieties**

| Sl.No. | Variety | *Incubation period(days) | **21 DAI | | | Symptom expression |
|---|---|---|---|---|---|---|
| | | | PDI | PDS | CI | |
| 1 | Aiswarya | 15 | 9.59[b] | 58.21[d] | 5.58[b] | Yellowing and wilting |
| 2 | Anashwara | 15 | 7.79[a] | 49.68[b] | 3.87[a] | Yellowing and wilting |
| 3 | Annapoorna | 15 | 7.51[a] | 56.20[c] | 4.22[a] | Yellowing and wilting |
| 4 | Aswathi | 15 | 7.00[a] | 29.00[a] | 2.03[a] | Yellowing and wilting |
| 5 | Bhadra | 15 | 9.27[a] | 66.29[e] | 6.14[c] | Yellowing and wilting |
| 6 | Bharathy | 15 | 9.66[c] | 49.93[b] | 4.82[a] | Yellowing and wilting |
| 7 | Harsha | 15 | 5.56[a] | 50.00[b] | 2.78[a] | Yellowing and wilting |
| 8 | Jaya | 15 | 7.48[a] | 59.33[d] | 4.43[a] | Yellowing and wilting |
| 9 | Jyothi | 15 | 11.11[d] | 50.67[c] | 5.63[b] | Yellowing and wilting |
| 10 | Kairali | 15 | 5.71[a] | 51.33[c] | 2.93[a] | Yellowing and wilting |
| 11 | Kanchana | 15 | 9.00[a] | 36.33[a] | 3.26[a] | Yellowing and wilting |
| 12 | Karuna | 15 | 8.63[a] | 43.00[a] | 3.71[a] | Yellowing and wilting |
| 13 | Kunju Kunju Varna | 15 | 7.92[a] | 48.95[b] | 3.87[a] | Yellowing and wilting |
| 14 | Manupriya | 15 | 8.22[a] | 59.33[d] | 4.87[a] | Yellowing and wilting |
| 15 | Matta Triveni | 15 | 8.67[a] | 34.00[a] | 2.94[a] | Yellowing and wilting |
| 16 | Neeraja | 15 | 7.44[a] | 31.00[a] | 2.30[a] | Yellowing and wilting |
| 17 | Rohini | 15 | 11.11[d] | 62.57[d] | 6.95[d] | Yellowing and wilting |
| 18 | Sabari | 15 | 9.48[b] | 58.67[d] | 5.56[b] | Yellowing and wilting |
| 19 | Swetha | 15 | 7.09[a] | 33.33[a] | 2.36[a] | Yellowing and wilting |
| 20 | Uma | 15 | 11.53[e] | 56.76[c] | 6.54[c] | Yellowing and wilting |

* Incubation period refers to the time taken (days) for expression of initial symptom

** Mean of five replications

Values under same subscript form a homogenous sub group

#### 4.5.3.14. Moncombu isolate (XMOU-1)

As presented in the Table 10.14, the Moncombu isolate could show the CI values ranged from 2.30 to 6.95 and grouped all the varieties as resistant. On infection, the 20 varieties showed the initial symptom of yellowing with water soaked brown lesion on fifteen days after sowing the treated seeds followed by inward rolling and wilting.

The cumulative results of study on kresek symptom development on 20 rice varieties, are presented in the Table11.The five isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed susceptible reaction on all 20 varieties. The seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) isolates showed moderately susceptible reaction on all the 20 varieties. The two isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed resistant reaction on all the 20 rice varieties.

### 4.5.4. Classification of different isolates of *Xoo* based on disease reaction

Based on the studies *viz.*, surveys in 14 locations, colony characters of the bacterial isolates, pathogenic variability on 20 varieties, near isogenic lines and rice differentials, the 14 isolates of *Xoo* were categorized as 'highly virulent', 'moderately virulent' and 'weakly virulent'.

#### 4.5.4.1. Characteristics of highly virulent isolates of *Xoo*

The characteristics of the five highly virulent isolates of *Xoo viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) are presented in Table.12. The field survey in rice growing areas of Alappuzha, Palakkad and Thrissur districts of Kerala covering 14 locations to study the occurrence of bacterial blight disease revealed that the five isolates of the pathogen from Palakkad district *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed very high PDI, PDS and CI values ranging from 47.55 – 60.34, 85.49 –

**Table 11. Studies on kresek reaction of fourteen *Xoo* isolates on twenty cultivated rice varieties**

| Variety / Isolates | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ | $V_8$ | $V_9$ | $V_{10}$ | $V_{11}$ | $V_{12}$ | $V_{13}$ | $V_{14}$ | $V_{15}$ | $V_{16}$ | $V_{17}$ | $V_{18}$ | $V_{19}$ | $V_{20}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XAMI-3 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| XERM-1 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| XKOR-3 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| XMRA-2 | MS | MS | MS | MS | S | MS | MS | S | MS | S | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XNRA-1 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| XPAI-3 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| XPTB-4 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XPLY-5 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| XAGA-2 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XKDA-1 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XMTY-2 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XEDA-3 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XKVA-1 | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS | MS |
| XMOU-1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |

$V_1$. Aiswarya $V_2$. Anashwara $V_3$. Annapoorna $V_4$. Aswathi $V_5$. Bhadra $V_6$. Bharathy $V_7$. Harsha $V_8$. Jaya V9. Jyothi $V_{10}$. Kairali $V_{11}$. Kanchana $V_{12}$. Karuna $V_{13}$. Kunju Kunju Varna $V_{14}$. Manupriya $V_{15}$. Matta Triveni $V_{16}$. Neeraja $V_{17}$. Rohini $V_{18}$. Sabari $V_{19}$. Swetha $V_{20}$. Uma

R- Resistant MS - Moderately susceptible S - Susceptible

## Table 12. Characteristics of highly virulent isolates of *Xoo*

| Sl.No. | Isolates | Field survey | | | Colony characters | | | Leaf blight reaction on cultivated varieties (Average over twenty varieties) | | | Leaf blight reaction on near isogenic lines/rice differentials (Average over nine lines / differentials ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PDI | PDS | CI values | Incubation period (h) | *Sliminess | Pigmentation | Incubation period (days) | CI values | Lesion length (cm) | Incubation period (days) | CI values | Lesion length (cm) |
| 1. | Athimani (XAMI-3) | 53.95 | 87.65 | 47.30 | 36 | +++ | Dark yellow | 2.65 | 42.72 | 16.40 | 2.00 | 25.17 | 11.42 |
| 2. | Erattakulam (XERM-1) | 60.34 | 92.42 | 55.77 | 48 | +++ | Dark yellow | 2.65 | 45.24 | 19.65 | 2.00 | 30.19 | 11.41 |
| 3. | Nenmara (XNRA-1) | 47.55 | 88.63 | 42.13 | 48 | +++ | Dark yellow | 2.65 | 40.71 | 15.25 | 2.00 | 29.68 | 11.17 |
| 4. | Parali (XPAI-3) | 53.87 | 87.18 | 46.96 | 48 | +++ | Dark yellow | 2.65 | 45.26 | 17.63 | 2.22 | 31.42 | 11.61 |
| 5. | Polpully (XPLY-5) | 49.77 | 85.49 | 42.55 | 36 | +++ | Dark yellow | 2.65 | 39.82 | 15.09 | 2.00 | 30.25 | 11.50 |

* +++ Good

92.42 and 42.13 – 55.77 respectively on the two popular varieties Jyothi (PTB-39) and Uma (MO -16). The colony characters of the pathogen isolated from the above places showed that they were dark yellow slimy colonies and the incubation period for colony development ranged from 36 – 48 h. The leaf blight reaction on 20 varieties, six near isogenic lines and three differentials showed that the pathogens from these places took a mean incubation period of 2.65 days to develop initial symptom on cultivated varieties and two days incubation period on six near isogenic lines/three differentials. The CI values on 20 varieties ranged from 39.82 to 45.26, where as on six near isogenic lines/three differentials ranged from 25.17 to 31.42.The lesion length on 20 varieties ranged from 15.25-19.65cm where as on six near isogenic lines/three differentials ranged from 11.17 to 11.61cm. Based on the above observations, the five isolates were classified as highly virulent.

#### 4.5.4.2. Characteristics of moderately virulent isolates of *Xoo*

The characteristics of the seven moderately virulent isolates *viz.*, Manchira (XMRA-4), Pattambi (XPTB-2), Akamala (XAKA-4), Kodakara (XKDA-3), Mannuthy (XMTY-2), Edathua (XEDA-3) and Karuvatta (XKVA-2) are presented in Table.13. The field survey conducted in rice growing areas of Alappuzha, Palakkad and Thrissur districts of Kerala covering 14 locations to study the occurrence of bacterial blight disease revealed that, seven pathogen population, two from Palakkad district *viz.*, Manchira (XMRA-4) and Pattambi (XPTB-2), three from Thrissur district *viz.*, Akamala (XAKA-4), Kodakara (XKDA-3) and Mannuthy (XMTY-2) two from Alappuzha district *viz.*, Edathua (XEDA-3) and Karuvatta (XKVA-2) showed the high PDI, PDS and CI vales ranging from 34.17 – 46.16, 73.31 – 85.72 and 27.26 – 39.33 respectively on the two popular varieties Jyothi (PTB-39) and Uma (MO-16). The colony characters of the pathogens from the above places showed that they were dark yellow and slimy except Edathua (XEDA-3), which was light yellow in colour and the incubation period for colony development was 24 h. The leaf blight reaction on 20 varieties, six near isogenic lines/three differentials showed that the pathogens from these places took a mean incubation period ranging from 3.65 to 3.70 days to develop initial symptom on cultivated varieties, and three days incubation period on six near isogenic lines/ three differentials. The CI values on 20 varieties ranged from 29.16 to 34.99, where as on six near

## Table 13. Characteristics of moderately virulent isolates of *Xoo*

| Sl. No. | Isolates | Field survey | | | Colony characters | | | Leaf blight reaction on cultivated varieties (Average over twenty varieties) | | | Leaf blight reaction on near isogenic lines/rice differentials (Average over nine lines / differentials ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PDI | PDS | CI values | Incubation period (h) | *Sliminess | Pigmentation | Incubation period(days) | CI values | Lesion length(cm) | Incubation period(days) | CI values | Lesion length(cm) |
| 1. | Manchira (XMRA-2) | 34.17 | 73.31 | 27.26 | 24 | +++ | Dark yellow | 3.65 | 30.91 | 11.78 | 3.00 | 21.21 | 7.86 |
| 2. | Pattambi (XPTB-4) | 42.95 | 85.72 | 36.80 | 24 | +++ | Dark yellow | 3.70 | 34.99 | 13.30 | 3.00 | 21.18 | 7.53 |
| 3. | Akamala (XAKA-2) | 41.55 | 83.21 | 34.61 | 24 | ++ | Light yellow | 3.70 | 33.12 | 12.56 | 3.00 | 17.23 | 7.71 |
| 4. | Kodakara (XKDA-1) | 44.83 | 83.43 | 37.40 | 24 | +++ | Dark yellow | 3.70 | 33.76 | 13.20 | 3.00 | 22.15 | 9.03 |
| 5. | Mannuthy (XMTY-2) | 43.62 | 81.93 | 35.72 | 24 | +++ | Dark yellow | 3.65 | 31.71 | 12.16 | 3.00 | 20.10 | 8.28 |
| 6. | Edathua (XEDA-3) | 46.16 | 85.18 | 39.33 | 24 | ++ | Light yellow | 3.70 | 34.81 | 13.36 | 3.00 | 18.43 | 8.17 |
| 7. | Karuvatta (XKVA-1) | 41.25 | 81.89 | 33.77 | 24 | +++ | Dark yellow | 3.70 | 29.16 | 12.18 | 3.00 | 20.95 | 8.37 |

* +++ Good; ++Moderate

isogenic lines/three differentials ranged from 17.23 to 22.15. They showed the lesion length on 20 varieties ranging from 11.78 to 13.36 cm, where as on six near isogenic lines/three differentials, it was from 7.53 to 9.03 cm. Based on the above observations, the seven isolates were classified as moderately virulent.

#### 4.5.4.3. Characteristics of weakly virulent isolates of *Xoo*

The characteristics of the two weakly virulent isolates *viz*., Kodallur (XMRA-4) and Moncombu (XMOU-1) are presented in Table.14. The field survey in rice growing areas of Alappuzha, Palakkad and Thrissur districts of Kerala covering 14 locations to study the occurrence of bacterial blight disease revealed that two isolates namely Kodallur (XKOR-3) from Palakkad district and Moncombu (XMOU-1) from Alappuzha district showed less PDI, PDS and CI vales ranging from 20.94–21.18, 57.05 – 57.95 and 11.97– 12.29 respectively on Uma (MO-16) variety. The colony characters of the isolates from the above two places showed that they were of good slimy to moderate slimy and dark yellow to light yellow in colour, and the incubation period for colony development was 24 h. The leaf blight reaction on 20 varieties, six near isogenic lines/three differentials showed that the pathogens from these places took a mean incubation period of 5.75 days to develop initial symptom on cultivated varieties and 7.00 days incubation period on six near isogenic lines/three differentials. The CI values on 20 varieties ranged from 9.57 to 9.92 where as on six near isogenic lines/three differentials ranged from 2.31 to 3.39. The lesion length on 20 varieties ranged from 5.57 to 5.67cm where as on six near isogenic lines/three differentials, it ranged from 1.55 to 2.19 cm. Based on the above observations, the two isolates were classified as weakly virulent.

### 4.6. Studies on the genetic variability of *Xoo* isolates from three districts of Kerala

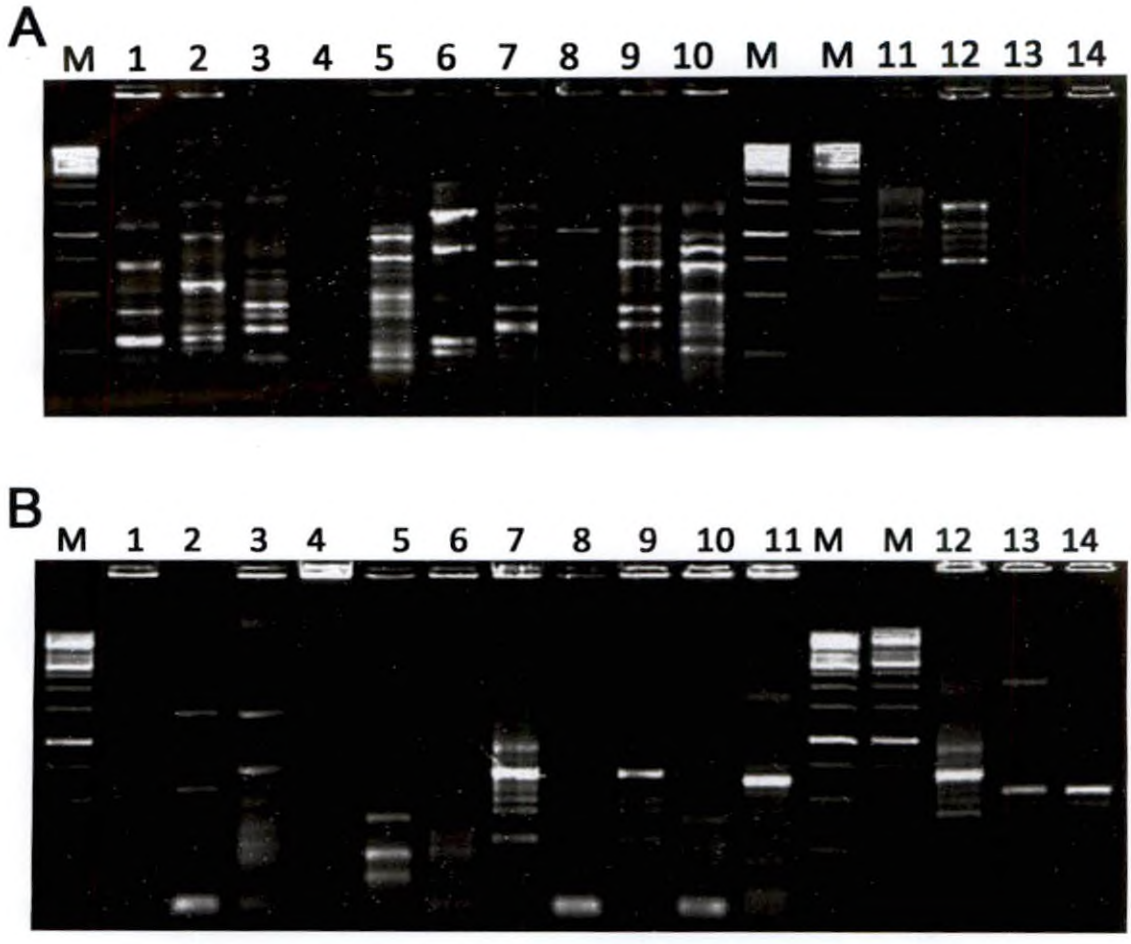
Genetic variability among the 14 *Xoo* isolates obtained from Alappuzha, Palakkad and Thrissur districts was assessed using the BOX and ERIC fingerprinting. The BOX-PCR fingerprinting could produce 5 to 14 bands per isolate with a size range of 100 bp to 2000 bp (Plate 5A). Among the isolates, Polpully isolate (X8) produced lowest amplicon numbers of 5, while Nenmara (X5) and Kodallur (X3) isolates produced 14 bands each. Likewise, ERIC

## Table 14. Characteristics of weakly virulent isolates of *Xoo*

| Sl.No. | Isolates | Field survey | | | Colony characters | | | Leaf blight reaction on cultivated varieties (Average over twenty varieties) | | | Leaf blight reaction on near isogenic lines/rice differentials (Average over nine lines / differentials ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PDI | PDS | CI values | Incubation period(h) | *Sliminess | Pigmentation | Incubation period(days) | CI values | Lesion length(cm) | Incubation period(days) | CI values | Lesion length(cm) |
| 1. | Kodallur (XKOR-3) | 21.18 | 57.95 | 12.29 | 24 | +++ | Dark yellow | 5.75 | 9.57 | 5.57 | 7.00 | 3.39 | 2.19 |
| 2. | Moncombu (XMOU-1) | 20.94 | 57.05 | 11.97 | 24 | ++ | Light yellow | 5.75 | 9.92 | 5.67 | 7.00 | 2.31 | 1.55 |

* +++ Good ; ++ Moderate

**Plate 5. A & B. Gel showing DNA fingerprints of *X. oryzae* pv. *oryzae* isolates with BOX and ERIC primers**

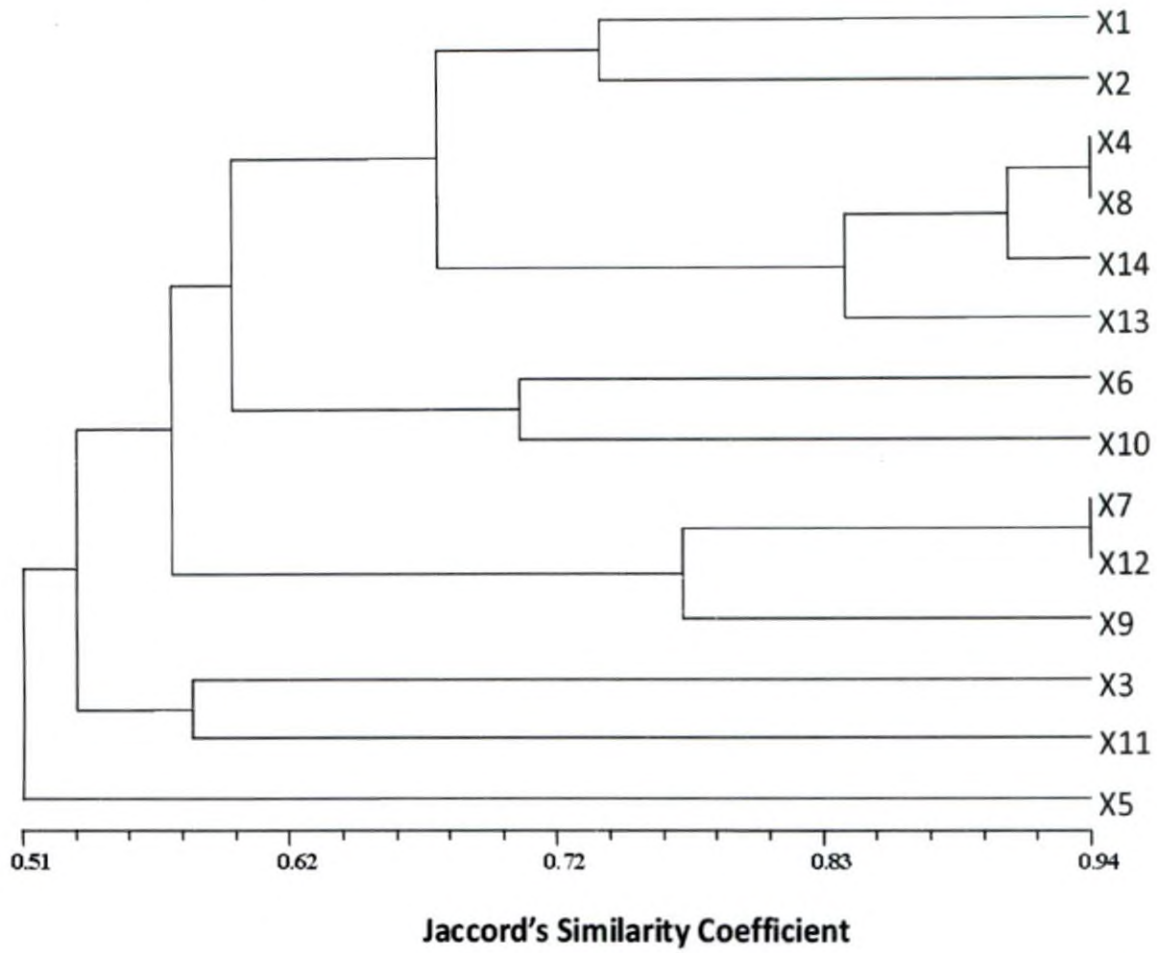



**BOX (A) and ERIC (B) fingerprinting of *Xanthomonas oryzae* pv. *oryzae* isolates,**

**M –1 kb DNA ladder; 1 to 14 –*X. oryzae* pv. *oryzae* isolates**

**(X1) Athimani (XAMI-3)**
**(X2) Erattakulam (XERM-1)**
**(X3) Kodallur (XKOR-3)**
**(X4) Manchira (XMRA-2)**
**(X5) Nenmara (XNRA-1)**
**(X6) Parali (XPAI-3)**
**(X7) Pattambi (XPTB-4)**
**(X8) Polpully (XPLY-5)**
**(X9) Akamala (XAKA-2)**
**(X10) Kodakara (XKDA-1)**
**(X11) Mannuthy (XMTY-2)**
**(X12) Edathua (XEDA-3)**
**(X13) Karuvatta (XKVA-1)**
**(X14) Moncombu (XMOU-1)**

**Fig 1. Dendrogram based on BOX and ERIC fingerprinting of *X. oryzae* pv. *oryzae* isolates**




**UPGMA dendrogram based on BOX and ERIC fingerprinting of *X. oryzae* pv. *oryzae* isolates.The cluster was built based on the matrix generated with DNA fragments amplified by BOX and ERIC-PCR. The coefficient used was Jaccard's similarity coefficient. X1-14 refers to *X. oryzae* pv. *oryzae* isolates.**

(X1) Athimani (XAMI-3)
(X2) Erattakulam (XERM-1)
(X3) Kodallur (XKOR-3)
(X4) Manchira (XMRA-2)
(X5) Nenmara (XNRA-1)
(X6) Parali (XPAI-3)
(X7) Pattambi (XPTB-4)
(X8) Polpully (XPLY-5)
(X9) Akamala (XAKA-2)
(X10) Kodakara (XKDA-1)
(X11) Mannuthy (XMTY-2)
(X12) Edathua (XEDA-3)
(X13) Karuvatta (XKVA-1)
(X14) Moncombu (XMOU-1)

fingerprinting could able to produce 4 to 10 amplicons with a range from 100 to 1500 bp (Plate 5B). Among the isolates, Karuvatta isolate (X13) produced less number of four bands, while Pattambi (X7) and Edathua (X12) isolates showed 10 bands each (Fig.).The Manchira isolate (X4) did not produce any bands in both BOX and ERIC-PCR.

When the binary data (presence or absence of bands at a particular size in an isolate) obtained from the BOX and ERIC-PCR banding patterns were used for grouping the isolates by UPGMA method, the dendrogram revealed that the isolates showed the similarity ranging from 50 to 94 per cent (Fig 1). When 80 per cent similarity was used as limit of discrimination, the isolates *viz*., Manchira (X4), Polpully (X8) and Moncombu (X14) clustered tightly showing more than 90 per cent similarity. Similarly, the isolates *viz*., Pattambi (X7) and Edathua (X12) showed more similarity (94%) and were tightly clustered. Remaining isolates *viz*., Athimani (X1), Erattakulam (X2), Kodallur (X3), Nenmara (X5), Parali (X6), Akamala (X9), Kodakara (X10), Mannuthy (X11) and Karuvatta (X13) showed very high variability (less than 80% similarity) within themselves and other isolates each other.

### 4.7. Management of bacterial blight of rice

#### 4.7.1. *In vitro* sensitivity of different bactericides against highly virulent isolates of *Xoo*

*In vitro* sensitivity of bactericides *viz*., tetracycline (50 ppm,100 ppm and 250 ppm), streptocycline (50 ppm, 200 ppm and 250 ppm), streptomycin sulphate (50 ppm, 100 ppm and 250 ppm), Bactrinashak (50 ppm, 100ppm and 250 ppm), ampicillin (50 ppm, 100 ppm and 250 ppm) and penicillin (50 ppm, 100 ppm and 250 ppm) against four highly virulent isolates of *Xoo viz*., Athimani (XAMI-3), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) was carried out. The results are presented in Table 15.

Among the bactericides tested at various concentrations against four virulent isolates, tetracycline 50 ppm, 100 ppm and 250 ppm showed mean per cent inhibition of 47.90, 52 and 62.86 respectively. The increased effect was proportional to the concentration (Fig 2) (Plate 6). Streptocycline 50 ppm and 100 ppm did not show any inhibition, but showed the inhibition at

**Table 15. *In vitro* sensitivity of different bactericides against highly virulent isolates of *Xoo***

| Sl. No. | Isolates / Bactericides | Athimani | Nenmara | Parali | Polpully | Mean |
|---|---|---|---|---|---|---|
| | | Per cent inhibition | Per cent inhibition | Per cent inhibition | Per cent inhibition | Per cent inhibition |
| 1. | Tetracycline 50 ppm | 49.12 | 50.00 | 46.25 | 46.25 | 47.90 |
| 2. | Tetracycline 100 ppm | 57.50 | 55.37 | 48.00 | 47.12 | 52.00 |
| 3. | Tetracycline 250 ppm | 68.75 | 64.32 | 60.50 | 57.87 | 62.86 |
| 4. | Streptocycline 200 ppm | 21.70 | 20.37 | 19.12 | 19.62 | 20.20 |
| 5. | Streptocycline 250 ppm | 23.75 | 24.12 | 23.12 | 13.75 | 21.18 |
| 6. | Bactrinashak 100 ppm | 8.36 | 8.37 | 8.75 | 8.75 | 8.55 |
| 7. | Bactrinashak 250 ppm | 20.37 | 23.70 | 18.75 | 18.85 | 20.42 |

CD for treatments = 1.06

**Plate 6. *In vitro* sensitivity of different bactericides against highly virulent isolates of *Xoo***

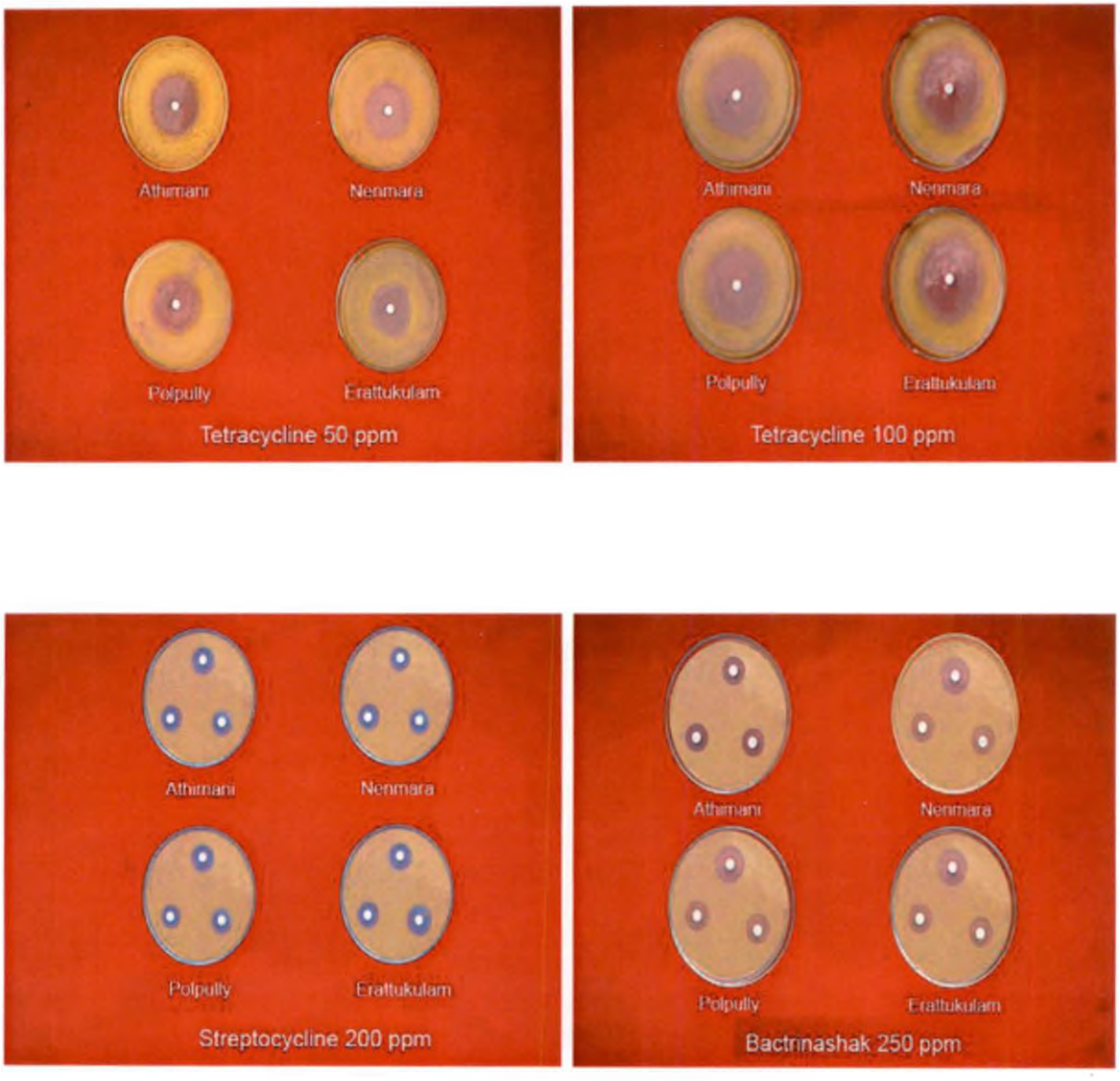
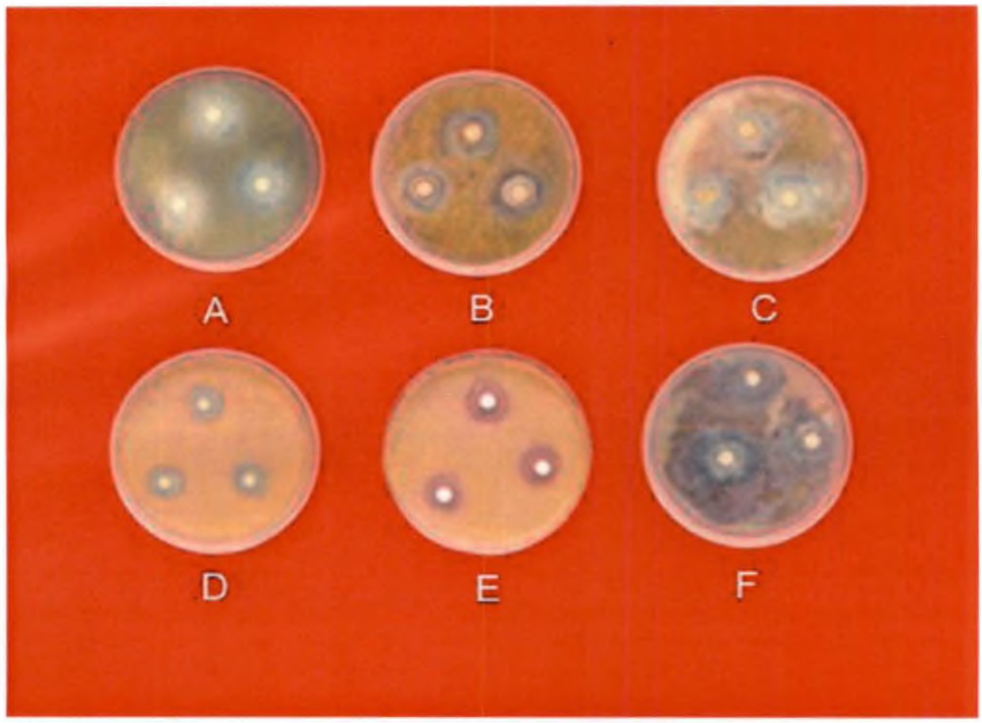


**Plate 7. *In vitro* sensitivity of different organics and agrochemicals against *Xoo***




A : *Pseudomonas fluorescens* 2%

B : Vermicompost extract 2% + *P. fluorescens* 2%

C : Cow dung extract 2% + *P.fluorescens* 2%

D : Vermicompost extract 2%

E : Copper Hydroxide 0.15%

F : Cow dung extract 2% + Vermicompost extract 2%

**Fig 2. *In vitro* sensitivity of bactericides against highly virulent isolates of *X.oryzae* pv. *oryzae***

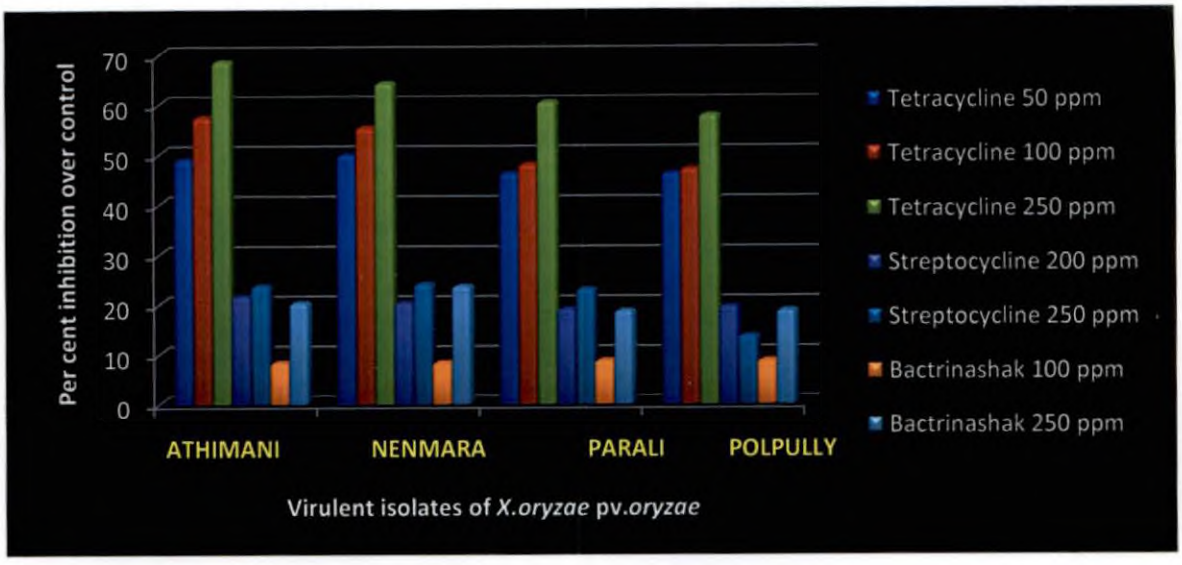
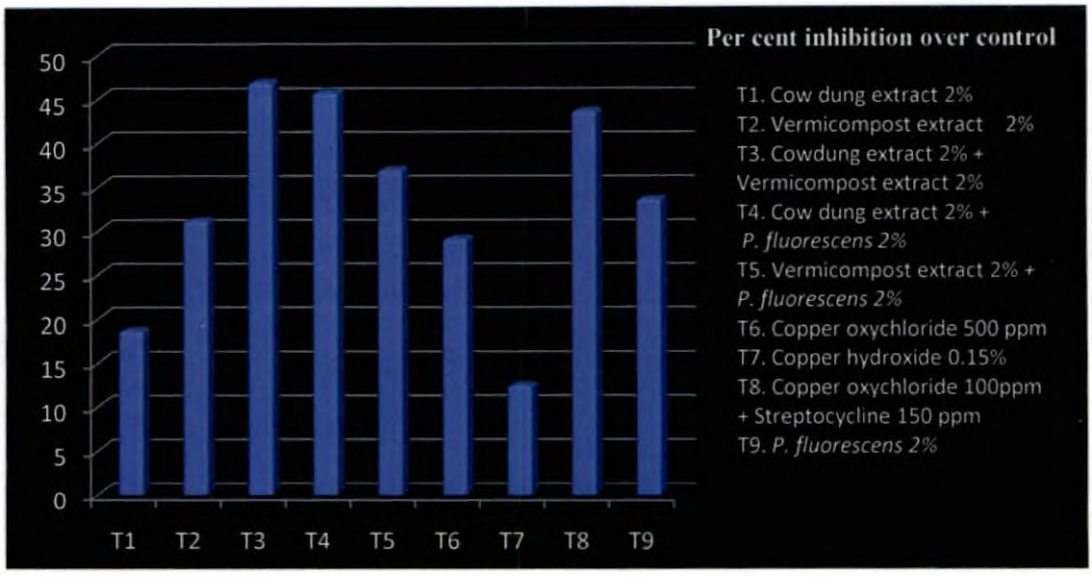



**Fig 3. *In vitro* sensitivity of organics and agrochemicals against *X.oryzae* pv.*oryzae***



200 ppm and 250 ppm and recorded the per cent inhibition of 20.20 and 21.18 respectively. Bactrinashak at 50 ppm did not show any inhibition but showed inhibition at 100 ppm and 250 ppm and recorded the per cent inhibition of 8.55 and 20.42 respectively. The other antibiotics *viz.*, streptomycin sulphate (50 ppm,100 ppm and 250 ppm), ampicillin (50 ppm,100 ppm and 250 ppm), penicillin (50 ppm,100 ppm and 250 ppm) were not found to inhibit *Xoo*.

### 4.7.2. *In vitro* sensitivity of different organics and agrochemicals against *Xoo*

*In vitro* sensitivity of different organics and agrochemicals *viz.*, cow dung extract 2%, vermicompost extract 2%, cow dung extract 2% + vermicompost extract 2%, cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2%, copper oxychloride 500 ppm, copper hydroxide 0.15%, copper oxychloride 500ppm + streptocycline 150 ppm and *Pseudomonas fluorescens* 2% were tried under *in vitro* against the highly virulent Polpully (XPLY-5) isolate of the *Xoo* and the results are presented in Table 16 (Plate 7). The study revealed that the cow dung extract 2% + vermicompost extract 2% showed maximum per cent inhibition of 47 which was found on par with cow dung extract 2% + *P.fluorescens* 2% (45.83). Vermicompost extract 2% + *P. fluorescens* 2% ranked next with per cent inhibition of 43.75 (Fig 3). Vermicompost extract 2 % alone found next with per cent inhibition of 37.08 and were on par with copper hydroxide 0.15% (33.75). Cow dung extract showed 29.16 per cent inhibition. Copper oxychloride 500 ppm + streptocycline 150 ppm showed the per cent inhibition of 18.75 and copper oxychloride 500 ppm alone showed a minimum per cent inhibition of 12.50.

### 4.7.3. Isolation and enumeration of rhizo and endosphere bacteria in rice

Fifty six samples of rice rhizosphere soil were collected from rice growing areas of Alappuzha, Palakkad and Thrissur districts. The total microflora *viz.*, fungi, bacteria and actinomycetes of the soil samples were quantitatively estimated by serial dilution plating technique.

From the data, it is evident that the rhizosphere population of microbes varied with different locations (Table 17a). The rhizosphere microbial population was maximum in soil

**Table16. *In vitro* sensitivity of different organics and agrochemicals against *Xoo***

| Sl. No. | Organics/agrochemicals | *Per cent inhibition |
|---|---|---|
| 1. | Cow dung extract 2% | $29.16^{d}$ |
| 2. | Vermicompost extract 2% | $37.08^{c}$ |
| 3. | Cow dung extract 2%+ vermicompost extract 2% | $47.00^{a}$ |
| 4. | Cow dung extract 2% + *Pseudomonas fluorescens* 2% | $45.83^{a}$ |
| 5.. | Vermicompost extract 2% + *P. fluorescens* 2% | $43.75^{b}$ |
| 6. | Copper oxychloride 500 ppm | $12.50^{e}$ |
| 7. | Copper hydroxide 0.15% | $33.75^{c}$ |
| 8. | Copper oxychloride 500 ppm + streptocycline 150 ppm | $18.75^{e}$ |
| 9. | *P. fluorescens* 2% | $31.25^{d}$ |

* Mean of three replications
Values under same subscript form a homogenous sub group

**Table 17a. Enumeration of total rhizosphere microflora from soil samples different locations**

| Sl. No. | Location | *Fungi (x $10^4$ cfu $g^{-1}$ soil) | *Bacteria ( x $10^7$ cfu $g^{-1}$ soil) | *Actinomycetes (x $10^5$ cfu $g^{-1}$ soil) |
|---|---|---|---|---|
| Rhizosphere microflora | | | | |
| 1. | PALAKKAD | | | |
| a. | Athimani | 29.50 | 37.75 | 5.50 |
| b. | Erattakulam | 30.75 | 33.25 | 11.5 |
| c. | Kodallur | 28.25 | 32.00 | 7.50 |
| d. | Parali | 22.00 | 28.00 | 10.25 |
| e. | Pattambi | 22.00 | 33.25 | 8.00 |
| f. | Manchira | 11.50 | 29.75 | 13.50 |
| g. | Nemmara | 42.25 | 65.00 | 22.50 |
| h. | Polpully | 18.50 | 34.00 | 7.25 |
| 2. | THRISSUR | | | |
| a. | Akamala | 23.25 | 28.75 | 8.00 |
| b. | Kodakara | 21.00 | 29.50 | 11.25 |
| c. | Mannuthy | 14.00 | 43.50 | 8.00 |
| 3. | ALAPPUZHA | | | |
| a. | Edathua | 13.50 | 32.75 | 8.00 |
| b. | Karuvatta | 16.75 | 37.50 | 8.75 |
| c. | Moncombu | 13.25 | 35.75 | 6.50 |

*(Mean of four replications)

samples from Nemmara of Palakkad district, than those from other locations of Thrissur and Alappuzha districts. Among the microflora, bacteria were more predominant in all soil samples followed by fungi and actinomycetes. The population of fungi showed variations among the different rhizosphere soil samples. The highest count of fungi 42.25 x $10^4$ cfu $g^{-1}$soil was observed from the Nenmara soils. The lowest count of 11.5x $10^4$ cfu $g^{-1}$ soil was in Manchira soil. The highest population of bacteria (65x$10^7$ cfu $g^{-1}$) was recorded in Nenmara soil of Palakkad district and the least (28x$10^7$ cfu $g^{-1}$) in Parali soil of Palakkad district. The population of actinomycetes varied with different locations with the maximum of 22.5 x $10^5$ cfu $g^{-1}$ soil in Nenmara soil and the minimum of 5.5 x $10^5$ cfu $g^{-1}$ soil from Athimani soil of Palakkad district.

Similarly, the endosphere microbial population also varied with different locations (Table17b).The endosphere microbial population was highest in samples from Kodallur area of Palakkad district, than those of other locations of Alapphuzha and Thrissur districts. Among the microflora, bacteria were more predominant in all the samples followed by actinomycetes and fungi. The endosphere fungal population showed variations among the different plant samples collected and ranged from 2.5 x $10^4$ cfu $g^{-1}$ to 11 x $10^4$ cfu $g^{-1}$.The highest count of fungi recorded from the Kodallur soil (11 x $10^4$ cfu $g^{-1}$).The lowest count of 2.5 x $10^4$ cfu $g^{-1}$ was recorded from the Manchira samples. The highest population of bacteria (19.5x$10^7$cfu $g^{-1}$) was recorded from Kodallur sample of Palakkad district and the least (6x$10^7$cfu $g^{-1}$) from Moncombu sample. The population of actinomycetes varied with different locations with the maximum of 6.75x$10^5$ cfu $g^{-1}$ from samples of Kodallur and the minimum of 4.75 x $10^5$ cfu $g^{-1}$ from samples of Akamala of Thrissur district.

The microbial population from cow dung and vermicompost from Pattambi samples indicated that both the sources had the highest bacterial count (Table 17c). The bacterial count ranged from 31.14- 33.80 x$10^7$ cfug$^{-1}$. Representative bacterial colonies obtained from the enumeration of total microflora of rhizosphere, endosphere, cowdung and vermicompost were selected. Further, isolation and selection of representative rhizosphere and endosphere bacterial colonies were carried out following serial dilution plate technique using Nutrient agar (NA), King's B, Soil extract agar (SEA), Methyl red (MR) and Crystal violet (CV) agar media.

17b. Enumeration of total endosphere microflora from plant samples of different locations

| Sl. No. | Location | *Fungi (x $10^4$ cfu $g^{-1}$) | *Bacteria (x $10^7$ cfu $g^{-1}$) | *Actinomycetes (x $10^5$ cfu $g^{-1}$) |
|---|---|---|---|---|
| **Endosphere microflora** | | | | |
| 1. | **PALAKKAD** | | | |
| a. | Athimani | 5.50 | 7.50 | 6.50 |
| b. | Erattakulam | 5.50 | 7.25 | 5.50 |
| c. | Kodallur | 11.00 | 19.50 | 6.75 |
| d. | Parali | 2.80 | 8.20 | 5.20 |
| e. | Pattambi | 3.25 | 7.75 | 5.75 |
| f. | Manchira | 2.50 | 8.25 | 5.00 |
| g. | Nemmara | 2.75 | 7.50 | 6.00 |
| h. | Polpully | 3.00 | 7.75 | 5.20 |
| **2.** | **THRISSUR** | | | |
| a. | Akamala | 4.25 | 7.00 | 4.75 |
| b. | Kodakara | 4.25 | 7.25 | 5.50 |
| c. | Mannuthy | 4.50 | 7.50 | 5.00 |
| **3.** | **ALAPPUZHA** | | | |
| a. | Edathua | 4.50 | 8.50 | 5.25 |
| b. | Karuvatta | 4.00 | 7.75 | 5.75 |
| c. | Moncombu | 4.25 | 6.00 | 5.25 |

(Mean of four replications)

17c. Enumeration of total microflora from cowdung and vermicompost samples

| Sl. No. | Location | *Fungi (x $10^4$ cfu $g^{-1}$) | *Bacteria (x $10^7$ cfu $g^{-1}$) | *Actinomycetes (x $10^5$ cfu $g^{-1}$) |
|---|---|---|---|---|
| **Cowdung and vermicompost microflora** | | | | |
| **Cowdung** | | | | |
| 1. | Pattambi | 6.80 | 33.80 | 5.00 |
| **Vermicompost** | | | | |
| 1. | Pattambi | 6.42 | 31.14 | 6.85 |

*(Mean of four replications)

Altogether, 110 bacterial isolates obtained from rhizosphere, endosphere, cow dung and vermicompost were selected and they were further purified following standard protocols and maintained in NA slants as well as in sterile water and stored at 4°C for further studies.

**4.7.3.1. Screening of bacterial isolates from rhizosphere, endosphere, cowdung and vermicompost against *Xoo***

Preliminary screening of 110 bacterial isolates obtained from rhizosphere, endosphere, cow dung and vermicompost were tested for their antagonistic activity against the highly virulent Polpully isolate (XPLY-5) by dual culture method. Out of the 110 bacterial isolates screened, 18 were found to be antagonistic to the pathogen and the remaining bacterial isolates had over grown on the pathogen. The cow dung bacterium (CB-39) from Pattambi had shown the maximum inhibition zone of 3cm followed by rhizosphere bacteria from Nenmara (RR-26) with the inhibition zone of 2.50cm. The vermicompost bacterial isolates (VB-67 and VB-69) from Pattambi with the inhibition zone of 2.30 cm each. A rhizosphere bacterium from Pattambi (RR-53) had shown the inhibition zone of 2.20 cm which was followed by the endosphere bacterium from Kodallur *viz.*, RE-1 with the inhibition zone of 2.10 (Table 18). Among the 18 isolates, five rhizobacteria, five endobacteria, five bacteria from cow dung and three bacteria from vermicompost were found to inhibit the pathogen and the mechanism of inhibition was lysis.

**4.7.3.2. *In vitro* evaluation of antagonistic activity of selected bacterial isolates against *Xoo***

The six bacterial isolates which showed high inhibition zone in the preliminary screening *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67 and VB-69 along with reference culture Pf1 were tested individually against the *Xoo* and the results are presented in Table 19. Among the different bacterial isolates, bacteria obtained from cow dung (CB-39) showed the maximum inhibition zone of 6.5 cm, followed by rhizosphere bacteria RR-26 with the inhibition zone of 6.2 cm. Bacteria from vermicompost *viz.*, VB-67 and VB-69 ranked next with the inhibition zone of 6 cm each. The reference culture Pf1 showed the inhibition zone of 5.5 cm. RR-53 and RE-1 showed the inhibition zone of 5 cm and 4.2 cm respectively (Plate 8). The antagonism

**Table 18.** ***In vitro*** **evaluation of bacterial isolates against the Polpully isolate of (*Xoo*)**

| Sl. No. | Isolate No. | Location | Host | Inhibition zone (cm) | Mechanism of antagonism |
|---|---|---|---|---|---|
| 1. | RR-20 | Athimani | Rice rhizosphere soil | 1.20 | Lysis |
| 2. | RR-26 | Nenmara | Rice rhizosphere soil | 2.50 | Lysis |
| 3. | RR-32 | Polpully | Rice rhizosphere soil | 1.00 | Lysis |
| 4. | RR-33 | Pattambi | Rice rhizosphere soil | 1.00 | Lysis |
| 5. | RR-53 | Pattambi | Rice rhizosphere soil | 2.10 | Lysis |
| 6. | RE-1 | Kodallur | Rice endophyte | 2.20 | Lysis |
| 7. | RE- 2 | Nenmara | Rice endophyte | 1.50 | Lysis |
| 8. | RE-3 | Pattambi | Rice endophyte | 1.50 | Lysis |
| 9. | RE-14 | Polpully | Rice endophyte | 0.50 | Lysis |
| 10. | RE-16 | Akamala | Rice endophyte | 1.00 | Lysis |
| 11. | CB-36 | Pattambi | Cow dung | 1.70 | Lysis |
| 12. | CB-39 | Pattambi | Cow dung | 3.00 | Lysis |
| 13. | CB-41 | Pattambi | Cow dung | 1.20 | Lysis |
| 14. | CB-52 | Pattambi | Cow dung | 1.50 | Lysis |
| 15. | CB-58 | Pattambi | Cow dung | 1.50 | Lysis |
| 16. | VB-67 | Pattambi | Vermicompost | 2.30 | Lysis |
| 17. | VB-69 | Pattambi | Vermicompost | 2.30 | Lysis |
| 18. | VB-70 | Pattambi | Vermicompost | 1.40 | Lysis |

**Table 19.** ***In vitro*** **evaluation of antagonistic activity of selected bacterial isolates against (*Xoo*)**

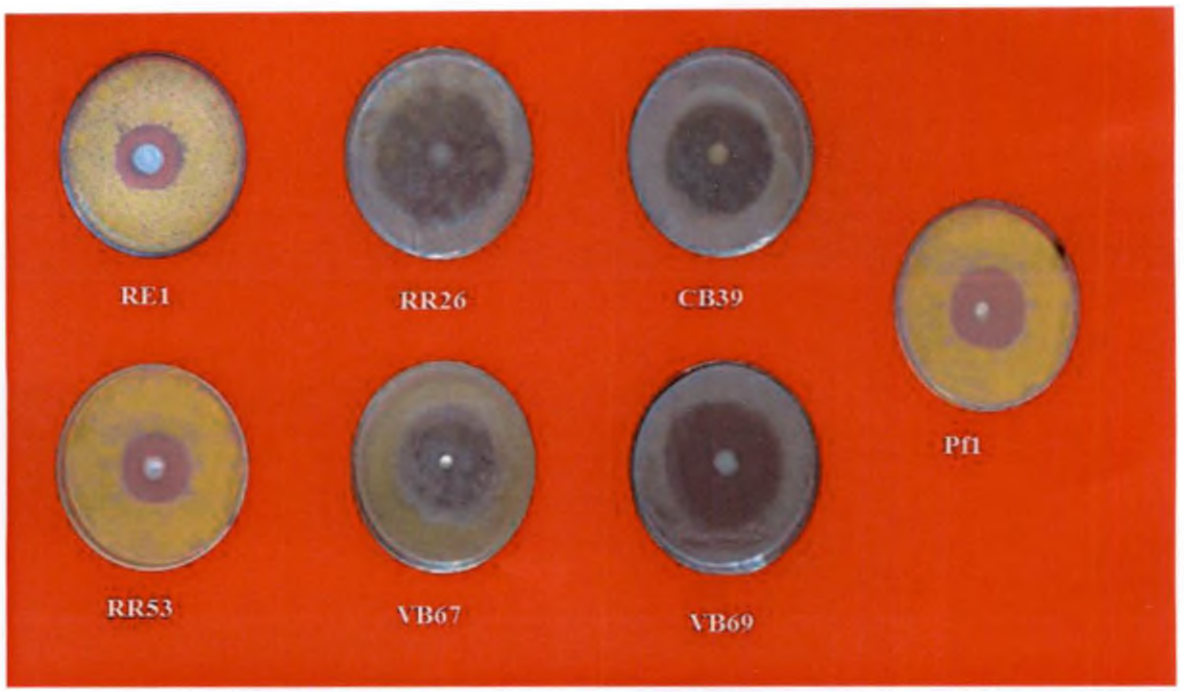
| Sl. No. | *Isolates | *Inhibition Zone (cm) | Score | * Per cent inhibition | Antagonism index |
|---|---|---|---|---|---|
| 1 | RE-1 | 4.2 | 4 | 52.50 | 2205.00 |
| 2 | RR-26 | 6.2 | 4 | 77.50 | 4805.00 |
| 3. | RR-53 | 5.0 | 4 | 62.50 | 3125.00 |
| 4. | CB-39 | 6.5 | 4 | 81.25 | 5281.25 |
| 5. | VB-67 | 6.0 | 4 | 75.00 | 4500.00 |
| 6. | VB-69 | 6.0 | 4 | 75.00 | 4500.00 |
| 7. | Pf-1 | 5.5 | 4 | 68.75 | 3781.25 |

*Mean of three replications

Scale of IZ : 1cm =1; 1-2cm =2; 2-3 cm =3; > 3 cm =4

*RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Plate 8. Antagonistic activity of selected bacterial isolates against *Xoo***




RE-1: Rice endosphere bacteria

RR-26: Rice rhizosphere bacteria

CB-39: Cowdung bacteria

RR-53: Rice rhizosphere bacteria

VB-67: Vermicompost bacteria

VB-69: Vermicompost bacteria

Pf-1: *P.fluorescens*

index (AI) was the highest for CB-39 (5281.25) followed by RR-26 (4805), VB-67 (4500), VB-69 (4500), Pf1 (3781.25), RR-53 (3125) and RE-1 (2205).

### 4.8. Cultural and morphological characteristics of promising bacterial isolates

On nutrient agar medium the bacterial isolates RE-1, RR-26, CB-39 and RR-53 showed 2 to 3 mm, white, circular shaped colonies, whereas VB-67 and VB-69 produced 2 to 3 mm, white to light brown, circular colonies. All the bacterial isolates were found to be short rods based on Gram's reaction (Table 20).

#### 4.8.1. Biochemical characterization of promising bacterial isolates

Biochemical characteristics of promising bacterial isolates were studied using HIMEDIA KB 002 Hi Assorted™ Biochemical Test Kit and other 11 biochemical tests. The results are presented in the Table 21.

The data revealed that, all the six bacterial isolates showed positive reaction for citrate utilization which was evident by the change of initial green colour of medium to blue colour. The isolate VB-67 showed positive reaction to Lysine decarboxylase where as RE-1, VB-67 and VB-69 showed positive reaction to ornithine decarboxylase. The isolates RR-53 and VB-69 showed positive reaction to urease test. Only VB-69 gave positive reaction to nitrate reduction test, where as RE-1 and VB-69 showed positive reaction to glucose utilization. The isolates RE-1, VB-67 and VB-69 showed positive response to adonitol utilization. The isolates RE-1, RR-53, VB-67 and VB-69 showed positive response to lactose utilization. All the six isolates showed positive response to arabinose utilization. Except VB-67, all the five isolates were found positive to sorbitol utilization test. All the six isolates produced pink colour rods on gram staining and found gram negative. All the six isolates could hydrolyse starch by producing colourless zone in contrast to the blue background of the medium around the bacterial growth on addition of iodine solution. The isolates RE-1, CB-39, VB-67 and VB-69 could liquefy gelatin. All the isolates produced white domed and mucoid colonies indicating positive reaction for levan production. All the six isolates showed pink colourisation to the medium showed their ability to hydrolyse arginine. All the isolates showed positive reaction to

**Table 20.Cultural and morphological characteristics of promising bacterial isolates**

| Sl.No. | Bacterial isolates | Cultural and morphological characters |
|---|---|---|
| 1. | RE-1 | 2 to 3 mm, white, circular colonies and rod shaped |
| 2. | RR-26 | 2 to 3 mm, white, circular colonies and rod shaped |
| 3. | RR-53 | 2 to 3 mm, white, circular colonies and rod shaped |
| 4. | CB-39 | 2 to 3 mm, white, circular colonies and rod shaped |
| 5. | VB-67 | 2 to 3 mm, white to light brown, circular colonies and rod shaped |
| 6. | VB-69 | 2 to 3 mm, white to light brown, circular colonies and rod shaped |

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria

**Table 21. Biochemical characterization of promising bacterial isolates**

| Biochemical tests | RE-1 | RR-26 | CB-39 | RR-53 | VB-67 | VB-69 |
|---|---|---|---|---|---|---|
| Citrate utllization | + | + | + | + | + | + |
| Lysine decarboxylase | - | - | - | - | + | - |
| Ornithine decarboxylase | + | - | - | - | + | + |
| Urease | - | - | - | + | - | + |
| Phenylalanine deamination | - | - | - | - | - | - |
| Nitrate reduction | - | - | - | - | - | + |
| $H_2S$ production | - | - | - | - | - | - |
| Glucose | + | - | - | - | - | + |
| Adonitol | + | - | - | - | + | + |
| Lactose | + | - | - | + | + | + |
| Arabinose | + | + | + | + | + | + |
| Sorbitol | + | + | + | + | - | + |
| Gram staining | -ve | -ve | -ve | -ve | -ve | -ve |
| Starch hydrolysis | + | + | + | + | + | + |
| Leven formation | + | + | + | + | + | + |
| Gelatin liquefaction | + | - | + | - | + | - |
| Arginine dihydrolase | + | + | + | + | + | + |
| VP test | - | - | - | - | - | - |
| MR test | - | - | - | - | - | - |
| 3% KOH solubility test | + | + | + | + | + | + |
| Oxidase test | - | - | - | - | - | - |
| Trytophan utilization | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + |
| Bacteria identified | *Pseudomonas* sp. | *Pseudomonas* sp. | *Pseudomonas* sp. | *Pseudomonas* sp. | *Pseudomonas* sp. | *Pseudomonas* sp. |

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria

KOH test by forming thick thread. This observation further confirmed the Gram negative reaction of these isolates. All the bacterial isolates showed positive reaction to Trytophan utilization. All the bacterial isolates produced effervescence when hydrogen peroxide was added to the cultures which indicated the production of catalase. Based on cultural, morphological and biochemical characters, these six Gram negative isolates tentatively were identified as *Pseudomonas* sp. and were selected for further studies.

### 4.9. *In planta* evaluation of antagonists, organics and agrochemicals against *Xoo*

A pot culture experiment was laid out for the evaluation of selected antagonists, antibiotics, fungicides and other organics *viz.*, cow dung extract and vermicompost extract against the highly virulent Polpully isolate (XPLY-5) of *Xoo*. Observations on per cent disease incidence (PDI), per cent disease severity (PDS) and coefficient of infection (CI) were calculated at 60 DAT. The results are presented in the Table 22 (Plate 9).

#### 4.9.1. Disease reaction due to various treatments

The data revealed that the PDI of various treatments varied from 33.33 to 100. Among the various treatments, tetracycline 250 ppm (T9) recorded the least per cent disease incidence (33.33) and was found to be superior followed by tetracycline 100 ppm (T8-39.99). The pots received rhizosphere bacteria RR-26 (T1), endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), tetracycline 50 ppm (T7), streptocycline 250 ppm (T11), Bactrinashak 250 ppm (T12), cow dung extract 2%+ vermicompost extract 2% (T15), cow dung extract 2% + *P. fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17) and KAU-Pfl (T20) ranked third with PDI value of 46.66. The treatments *viz.*, rhizosphere bacteria RR-53 (T2), streptocycline 200 ppm (T10), cow dung extract 2 % (T13) and vermicompost extract 2% (T14) recorded the PDI value of 53.32 and were on par, where as the treatment bacteria from vermicompost (T5) and copper hydroxide (0.15%) treated plants recorded the PDI value of 59.99. The pots which received the treatment copper oxychloride 500 ppm (T18) was the least effective and was on par with the absolute control (T21).

**Table 22.** ***In planta*** **evaluation of antagonists, organics and agrochemicals against** ***Xoo***

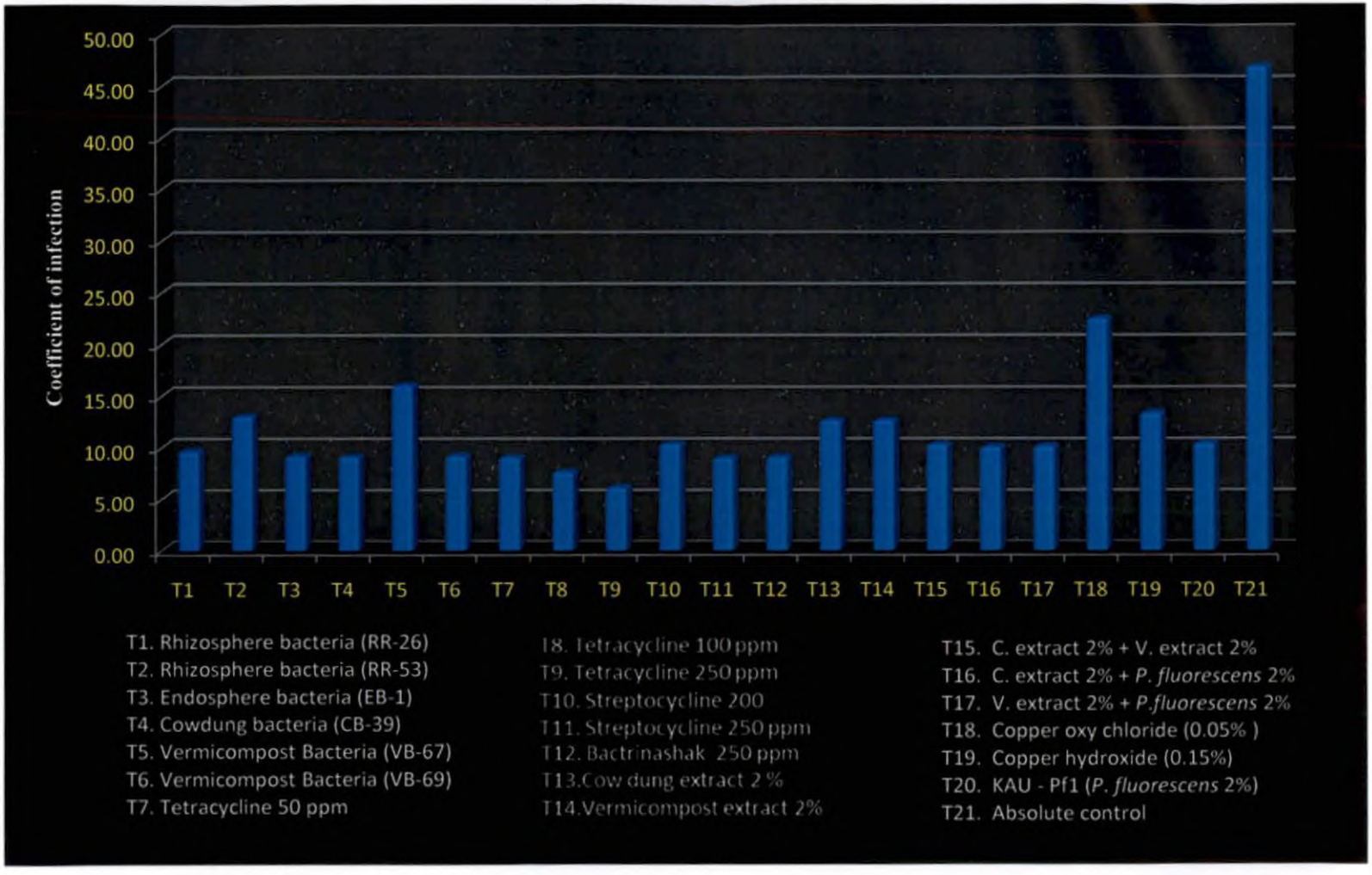
| Treatments | Disease reaction at 60 DAT | | |
|---|---|---|---|
| | PDI | PDS | CI |
| $T_1$. Rhizosphere bacteria (RR-26) | $46.66^{a}$ | $20.73^{b}$ | $9.67^{a}$ |
| $T_2$. Rhizosphere bacteria (RR-53) | $53.22^{a}$ | $24.50^{d}$ | $13.04^{b}$ |
| T3. Endosphere bacteria (RE-1) | $46.66^{a}$ | $19.78^{a}$ | $9.22^{a}$ |
| T4. Cow dung bacteria (CB-39) | $46.66^{a}$ | $19.58^{a}$ | $9.13^{a}$ |
| T5. Vermicompost bacteria (VB-67) | $59.99^{a}$ | $26.77^{e}$ | $16.05^{c}$ |
| T6. Vermicompost bacteria (VB-69) | $46.66^{a}$ | $19.73^{a}$ | $9.21^{a}$ |
| T7. Tetracycline 50 ppm | $46.66^{a}$ | $19.43^{a}$ | $9.06^{a}$ |
| T8. Tetracycline 100 ppm | $39.99^{a}$ | $19.21^{a}$ | $7.68^{a}$ |
| T9. Tetracycline 250 ppm | $33.33^{a}$ | $18.51^{a}$ | $6.16^{a}$ |
| T10. Streptocycline 200 ppm | $53.32^{a}$ | $19.48^{a}$ | $10.38^{a}$ |
| T11. Streptocycline 250 ppm | $46.66^{a}$ | $19.35^{a}$ | $9.03^{a}$ |
| T12. Bactrinashak 250 ppm | $46.66^{a}$ | $19.45^{a}$ | $9.08^{a}$ |
| T13. Cow dung slurry 2 % | $53.32^{a}$ | $23.70^{d}$ | $12.63^{a}$ |
| T14. Vermicompost slurry 2% | $53.32^{a}$ | $23.81^{d}$ | $12.69^{a}$ |
| T15. C. slurry 2% + V. slurry 2% | $46.66^{a}$ | $22.14^{c}$ | $10.33^{a}$ |
| T16. C. slurry 2% + *P. fluorescens* 2% | $46.66^{a}$ | $21.61^{c}$ | $10.08^{a}$ |
| T17. V. slurry 2% + *P. fluorescens* 2% | $46.66^{a}$ | $21.78^{c}$ | $10.16^{a}$ |
| T18. Copper oxychloride 500 ppm | $86.66^{b}$ | $25.98^{d}$ | $22.51^{d}$ |
| T19. Copper hydroxide (0.15%) | $59.99^{a}$ | $22.28^{c}$ | $13.36^{b}$ |
| T20. KAU - Pf1(*P. fluorescens* 2% standard check) | $46.66^{a}$ | $22.38^{c}$ | $10.44^{a}$ |
| T21. Absolute control | $100.00^{b}$ | $46.89^{f}$ | $46.89^{e}$ |

Values under same subscript form a homogenous sub group

RE- Rice endosphere bacteria
CB- Cow dung bacteria
Pf1 – *Pseudomonas fluorescens*
RR- Rice rhizosphere bacteria
VB- Vermicompost bacteria

**Plate 9. View of the pot culture experiment**

**Fig 4. *In planta* evaluation of antagonists, organics and agrochemicals for the management of bacterial blight (Coefficient of infection)**



The percent disease severity (PDS) varied from 18.51 to 46.89. All the treatments were significantly effective in managing the disease than the absolute control. Among the various treatments, the pots which received tetracycline 250 ppm (T9) ranked first with PDS value of 18.51 followed by tetracycline 100 ppm (T8) with PDS value of 19.21 were found to be highly effective in managing the disease. The treatments *viz.*, streptocycline 250 ppm (T10), tetracycline 50 ppm (T7), Bactrinashak 250 ppm (T12), streptocycline 200 ppm (T10), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6) and endosphere bacteria RE-1 (T3) were also found to be effective and were on par in checking the disease. They showed the PDS value of 19.35, 19.43, 19.45, 19.48, 19.58, 19.73 and 19.78 respectively. The pots treated with rhizosphere bacteria RR-26 (T1) showed the PDS value of 20.73 and found next in reducing the disease severity. The other treatemts *viz.*, cow dung extract 2% + *P.fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17), cow dung extract 2% + vermicompost extract 2% ( T15), copper hydroxide 0.15% (T19) and KAU-Pf1 (T20) were found on par with PDS value of 21.61, 21.78 , 22.14, 22.28 and 22.38 respectively. The treatments *viz.*, cow dung extract 2 % (T13), vermicompost extract 2% (T14), rhizosphere bacteria (RR-53) and copper oxychloride 500 ppm (T18) were found next in reducing the disease severity with the PDS value of 23.70, 23.81, 24.50 and 25.98 respectively. The treatment, bacteria from vermicompost VB-67 (T5) showed the per cent disease severity value of 26.77 where as the absolute control recorded the maximum PDS value of 46.89.

The CI values among the treatments varied from 7.68 to 46.89. All the treatments were found to be significantly effective in managing the disease than absolute control (Fig 4). Among the various treatments, tetracycline 250 ppm (T9) showed the minimum CI value of 6.16 and was found to be superior in checking the disease followed by the other treatments *viz.*, tetracycline 100 ppm (T8), streptocycline 250ppm (T11), Bactrinashak 250 ppm (T12) (Plate 10), tetracycline 50 ppm (T7), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), endosphere bacteria RE-1 (T3), rhizosphere bacteria RR-26 (T1) (Plate 11), cow dung extract 2% + *P.fluorescens* 2% (T17), vermicompost extract 2% + *P.fluorescens* 2% (T17) and cow dung extract 2% + vermicompost extract 2% (T15) (Plate 12) with the CI value of 7.68, 9.03, 9.06, 9.08, 9.13, 9.22, 9.67, 10.08, 10.16, 10.33, 12.63 and 12.69 respectively.The treatments *viz.*, rhizosphere bacteria RR-53 (T2) and the plants which

**Plate 10. Effect of selected treatments (T1, T4,T6) in managing bacterial blight**

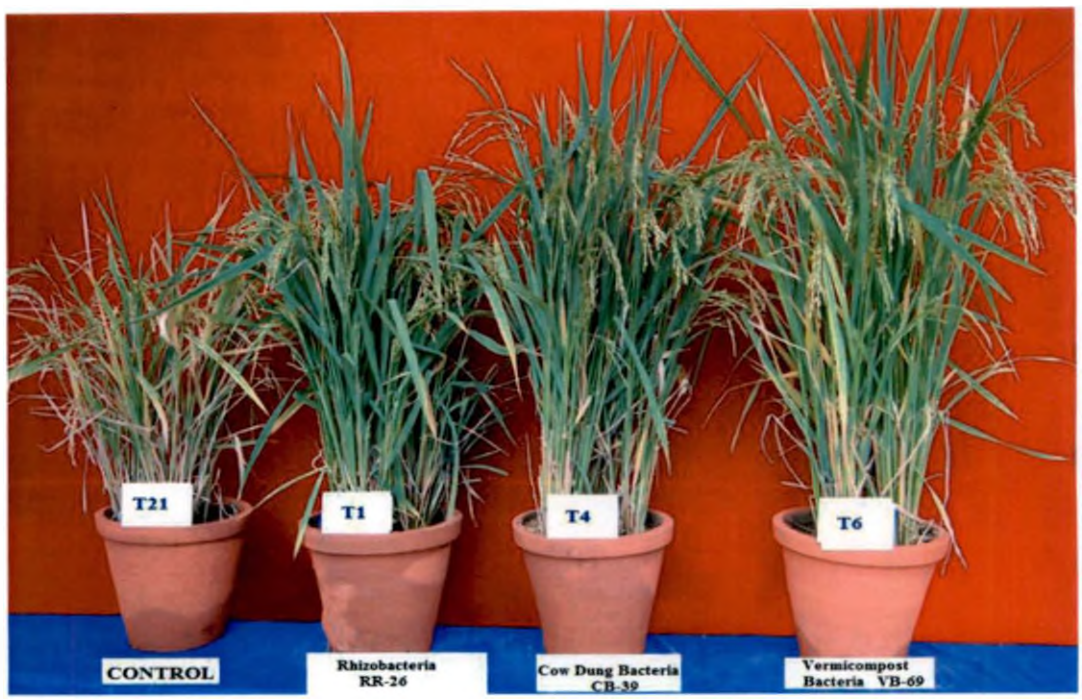



**Plate 11. Effect of selected treatments (T15, T16,T17) in managing bacterial blight**

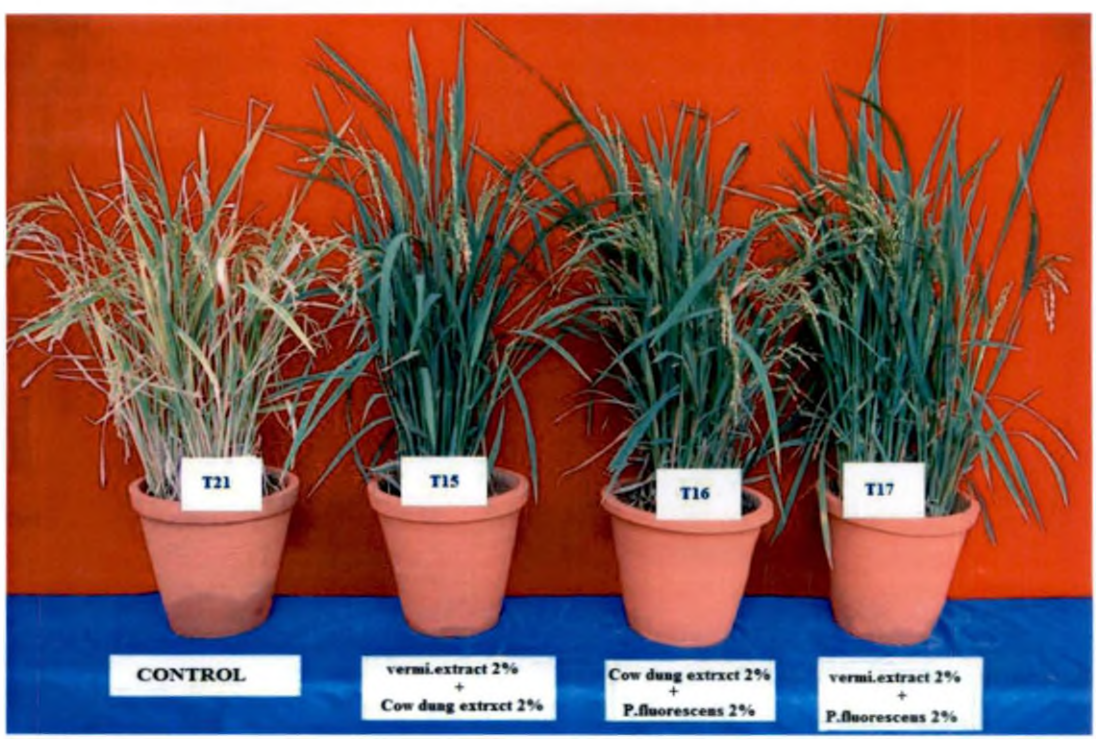
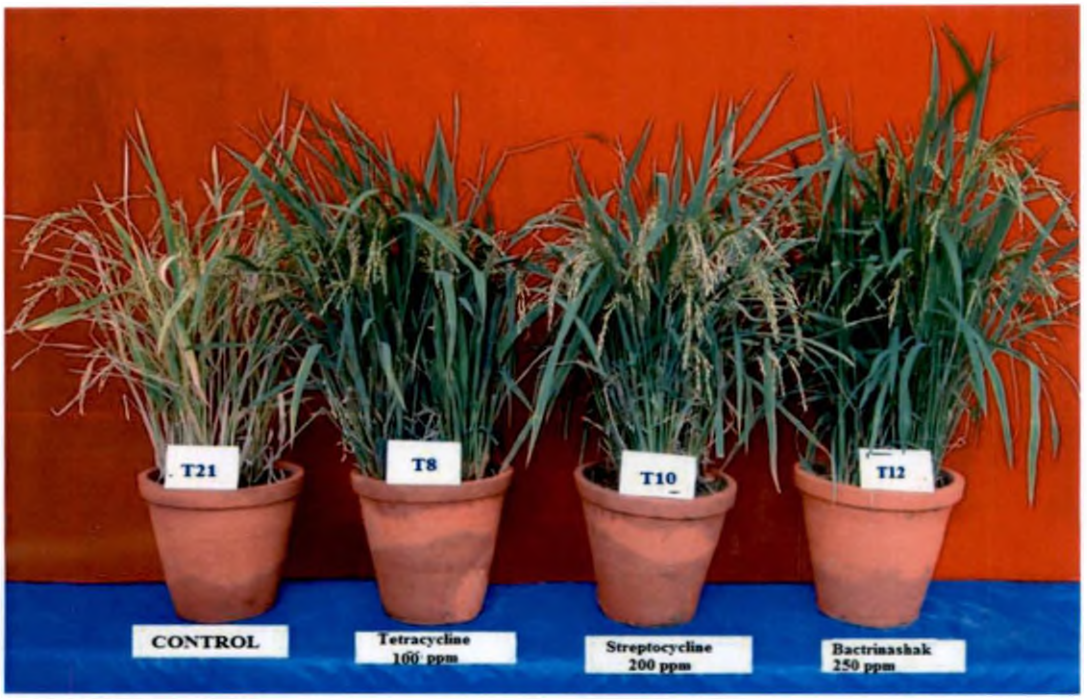


**Plate 12. Effect of selected treatments (T8, T10, T12) in managing bacterial blight**



received copper hydroxide 0.15% (T19) ranked next in checking the disease with CI value of 13.04 and 13.36 respectively and were on par with each other. The effectiveness of bacteria from vermicompost VB-67 (T5), copper oxychloride 500 ppm (T18) with the CI value of 16.05 and 22.51 respectively were also noticed. These two treatments were significantly different from each other but were superior to the control where the CI value in control pot was 46.89.

### 4.9.2. Effect of various treatments on biometric characters of rice plants

The effect of different treatments on biometric characters was studied and the results are presented in the Table 23.

#### 4.9.2.1. Growth characters

##### 4.9.2.1.1. Plant height (cm)

The plants which received treatments *viz.*, rhizosphere bacteria RR-26 (T1), endosphere bacteria RE-1 (T3), vermicompost extract 2% + *P. fluorescens* 2% (T17), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), KAU-Pf1 (T20), rhizosphere bacteria RR-53 (T2) and cow dung extract 2% + vermicompost extract 2% (T15) were showed the mean plant height of 88.82 cm, 88.16 cm, 87.74 cm, 87.24 cm, 83.70 cm, 83.70 cm, 83.21 cm, 81.38 cm and 80.48 cm respectively and found to be superior among the treatments. Bacterial antagonist obtained from vermicompost VB-67(T5) ranked next with the mean plant height of 78.15 cm. The treatments *viz.*, tetracycline 250 ppm (T9), Bactrinashak 250 ppm (T12), tetracycline100 ppm (T8) and tetracycline 50 ppm (T7) were found on par. They showed the mean plant height of 77.92cm, 77.62cm, 77.52cm and 77.36 cm respectively. The treatments *viz.*, vermicompost extract 2% (T14), streptocycline 250 ppm (T11), copper hydroxide 0.15% (T19) and cow dung extract 2 % (T13) were found on par. They showed the mean plant height of 76.46cm, 75.48cm, 74.96cm and 73.65cm respectively. The treatments *viz.*, copper oxychloride 500 ppm (T18) and absolute control (T21) were found on par. They showed the mean plant height of 63.74cm, 62.65cm and 57.65cm respectively.

Table 23. *In planta* evaluation of antagonists, organics and agrochemicals on biometric characters

| Treatments | Plant height (cm) | No. of productive tillers | Root length (cm) | Root wt. (g) | Panicle length (cm) | No.of filled grains/ Panicle | 1000 seed wt. (g) | Pot yield (g) | Straw yield (g) |
|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | $88.82^{a}$ | $15.30^{a}$ | $18.02^{a}$ | $13.50^{a}$ | $24.74^{a}$ | $81.56^{b}$ | $27.44^{a}$ | $86.60^{c}$ | $88.80^{a}$ |
| $T_2$ | $81.38^{a}$ | $15.67^{a}$ | $17.04^{a}$ | $12.98^{a}$ | $24.00^{a}$ | $82.06^{a}$ | $27.30^{a}$ | $86.83^{c}$ | $87.20^{a}$ |
| $T_3$ | $88.16^{a}$ | $15.62^{a}$ | $16.30^{a}$ | $12.01^{a}$ | $24.20^{a}$ | $80.64^{b}$ | $27.41^{a}$ | $87.60^{c}$ | $87.50^{a}$ |
| $T_4$ | $83.70^{a}$ | $15.67^{a}$ | $17.74^{a}$ | $12.90^{a}$ | $24.30^{a}$ | $80.86^{b}$ | $27.00^{a}$ | $87.11^{c}$ | $87.10^{a}$ |
| $T_5$ | $78.15^{b}$ | $10.31^{c}$ | $15.59^{b}$ | $11.06^{b}$ | $22.20^{b}$ | $69.92^{d}$ | $23.60^{c}$ | $72.12^{e}$ | $79.00^{b}$ |
| $T_6$ | $83.70^{a}$ | $16.07^{a}$ | $16.38^{a}$ | $13.16^{a}$ | $24.74^{a}$ | $82.84^{a}$ | $27.39^{a}$ | $88.21^{c}$ | $86.80^{a}$ |
| $T_7$ | $77.36^{c}$ | $15.92^{a}$ | $15.08^{c}$ | $9.30^{d}$ | $24.10^{a}$ | $85.32^{a}$ | $27.02^{a}$ | $94.07^{b}$ | $87.00^{a}$ |
| $T_8$ | $77.52^{c}$ | $15.99^{a}$ | $15.20^{c}$ | $9.82^{d}$ | $24.55^{a}$ | $90.32^{a}$ | $27.42^{a}$ | $96.46^{b}$ | $86.60^{a}$ |
| $T_9$ | $77.92^{c}$ | $16.08^{a}$ | $15.28^{b}$ | $10.14^{c}$ | $24.86^{a}$ | $95.44^{a}$ | $27.55^{a}$ | $109.23^{a}$ | $87.80^{a}$ |
| $T_{10}$ | $73.65^{d}$ | $10.80^{c}$ | $14.48^{d}$ | $6.96^{d}$ | $22.16^{b}$ | $80.68^{b}$ | $27.01^{a}$ | $84.85^{c}$ | $70.61^{c}$ |
| $T_{11}$ | $75.48^{d}$ | $15.52^{a}$ | $15.00^{c}$ | $8.42^{d}$ | $24.28^{a}$ | $82.80^{a}$ | $27.20^{a}$ | $86.46^{c}$ | $79.95^{b}$ |
| $T_{12}$ | $77.62^{c}$ | $15.33^{a}$ | $15.58^{b}$ | $9.80^{d}$ | $24.46^{a}$ | $80.24^{b}$ | $27.31^{a}$ | $84.67^{c}$ | $80.40^{b}$ |
| $T_{13}$ | $74.96^{d}$ | $11.40^{b}$ | $15.12^{c}$ | $9.82^{d}$ | $22.52^{b}$ | $76.32^{c}$ | $26.30^{b}$ | $71.79^{d}$ | $70.60^{c}$ |
| $T_{14}$ | $76.46^{d}$ | $11.78^{b}$ | $16.24^{b}$ | $9.78^{d}$ | $22.66^{b}$ | $76.88^{b}$ | $26.00^{b}$ | $71.39^{d}$ | $69.60^{c}$ |
| $T_{15}$ | $80.48^{a}$ | $11.17^{b}$ | $17.08^{a}$ | $13.07^{a}$ | $24.08^{a}$ | $86.04^{a}$ | $27.21^{a}$ | $84.68^{c}$ | $79.80^{b}$ |
| $T_{16}$ | $87.24^{a}$ | $15.47^{a}$ | $17.98^{a}$ | $13.48^{a}$ | $24.35^{a}$ | $86.70^{a}$ | $27.40^{a}$ | $86.05^{c}$ | $86.81^{a}$ |
| $T_{17}$ | $87.74^{a}$ | $15.58^{a}$ | $17.70^{a}$ | $13.38^{a}$ | $24.20^{a}$ | $85.92^{a}$ | $27.43^{a}$ | $84.01^{c}$ | $87.60^{a}$ |
| $T_{18}$ | $62.65^{e}$ | $7.44^{d}$ | $13.50^{d}$ | $6.12^{d}$ | $20.00^{c}$ | $65.44^{c}$ | $21.30^{d}$ | $43.13^{g}$ | $52.50^{d}$ |
| $T_{19}$ | $75.14^{d}$ | $10.16^{c}$ | $14.36^{d}$ | $8.27^{d}$ | $22.48^{b}$ | $76.42^{b}$ | $24.00^{c}$ | $59.72^{f}$ | $69.20^{c}$ |
| $T_{20}$ | $83.12^{a}$ | $15.10^{a}$ | $17.10^{a}$ | $12.00^{a}$ | $24.10^{a}$ | $79.94^{b}$ | $27.10^{a}$ | $85.77^{c}$ | $86.10^{a}$ |
| $T_{21}$ | $57.65^{e}$ | $5.66^{e}$ | $12.74^{d}$ | $5.72^{d}$ | $17.34^{d}$ | $59.34^{f}$ | $21.00^{d}$ | $35.55^{g}$ | $50.40^{d}$ |

Values under same subscript form a homogenous sub group

#### 4.9.2.1.2. Number of productive tillers

The effect of different treatments on mean number of productive tillers showed that the plants treated with the treatments *viz.*, tetracycline 250 ppm (T9), bacteria from vermicompost VB-69 (T6), tetracycline 100 ppm (T8), tetracycline 50 ppm (T7), bacteria from cow dung CB-39 (T4), rhizosphere bacteria RR-53 (T2), endosphere bacteria RE-1 (T3), vermicompost extract 2% + *P. fluorescens* 2% (T17), streptocycline 250 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T16), Bactrinashak 250 ppm (T12), rhizosphere bacteria RR-26 (T1) and KAU-Pfl(T20) were found significantly superior in increasing the number of productive tillers. They showed the mean productive tiller number of 16.08, 16.07, 15.99, 15.92, 15.67, 15.67, 15.62, 15.58, 15.52, 15.47, 15.33, 15.30 and 15.10 respectively. The treatments *viz.*, vermicompost extract 2% (T14), cow dung extract 2 % (T13) and cow dung extract 2%+ vermicompost extract 2% (T15) were found next in increasing the number of productive tillers. They showed the mean tiller number of 11.78, 11.40 and 11.17 respectively. The treatments *viz.*, streptocycline 200 ppm (T10) and cow dung extract 2% + vermicompost extract 2% (T15) were found on par. They showed the mean tiller number of 10.16 and 9.97 respectively. The treatments *viz.*, bacteria from vermicompost VB-67 (T5), streptocycline 200 ppm (T10) and copper hydroxide (0.15%) (T19) were found on par. They showed the mean tiller number of 10.31, 10.80 and 10.16 respectively. The treatment copper oxychloride 500 ppm (T19) showed the mean tiller number of 7.44 where as the absolute control showed the mean tiller number of 5.66.

#### 4.9.2.1.3. Root length (cm)

The effect of different treatments on mean root length showed that the plants which received the treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P.fluorescens* 2% (T16), bacteria from cow dung CB-39 (T4), vermicompost extract 2% + *P.fluorescens* 2% (T17), KAU-Pfl (T20), cow dung extract 2%+ vermicompost extract 2% (T15), rhizosphere bacteria RR-53 (T2) and tetracycline 100 ppm (T8) were found significantly superior and were on par in increasing the root length. They showed the mean root length of 18.02 cm, 17.98 cm, 17.74 cm, 17.70 cm, 17.10 cm, 17.08 cm, 17.04 cm and 16.30 cm

respectively. The treatments *viz.*, vermicompost extract 2% (T14), bacteria from vermicompost VB-67 (T5) and Bactrinashak 250 ppm (T12) were ranked next. They showed the mean root length of 16.24 cm, 15.59 cm and 15.58 cm respectively. The treatments *viz.*, tetracycline 100 ppm (T8), cow dung extract 2 % (T13), tetracycline 50 ppm (T7) and streptocycline 250 ppm (T11) were found on par. They showed the mean root length of 15.20 cm, 15.12 cm, 15.08 cm and 15.00 cm respectively. The treatments *viz.*, streptocycline 200 ppm (T10), copper hydroxide 0.15% (T19), copper oxychloride 500 ppm (T18) and absolute control were found on par. They showed the mean root length of 14.48 cm, 14.36 cm, 13.50 cm and 12.74 cm respectively.

**4.9.2.1.4. Root weight (g)**

The effect of different treatments on mean root weight showed that the plants which received the treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P.fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17), bacteria from vermicompost VB-69 (T6), cow dung extract 2% + vermicompost extract 2% (T15), rhizosphere bacteria RR-53 (T2), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1 (T3) and KAU-Pf1 (T20) were found significantly on par in increasing the root weight. They showed the mean root weight of 13.50g, 13.48g, 13.38g, 13.16g, 13.07g, 12.98g, 12.90g, 12.01g and 12.00 g respectively. The treatment bacteria from vermicompost VB-67 (T5) stood next. It showed the mean root weight of 11.06g. The treatment tetracycline 250 ppm (T9) showed the mean root weight of 10.14g. The treatments *viz.*, tetracycline 100 ppm (T8), Bactrinashak 250 ppm (T12), cow dung extract 2 % (T13), vermicompost extract 2% (T14), rhizosphere bacteria RR-53 (T2), streptocycline 250 ppm (T11), tetracycline 50 ppm (T7), copper hydroxide 0.15% (T20) and absolute control (T21) were found on par. They showed the mean root weight of 9.82g, 9.80g, 9.83g, 9.78g, 9.30g, 8.42g, 8.27g and 5.72 g respectively.

**4.9.2.2. Yield attributes**

**4.9.2.2.1. Panicle length (cm)**

The effect of different treatments on mean panicle length showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), vermicompost bacteria VB-69 (T6),

rhizosphere bacteria RR-26 (T1), tetracycline 100 ppm (T8), Bactrinashak 250 ppm (T12), cow dung extract 2% + *P.fluorescens* 2% (T17), bacteria from cow dung CB-39 (T4), streptocycline 250 ppm (T11), vermicompost extract 2% + *P. fluorescens* 2% (T18), endosphere bacteria RE-1 (T3), tetracycline 50 ppm (T7), cow dung extract 2%+ vermicompost extract 2% (T15), KAU-Pf1(T20) and rhizosphere bacteria RR-53 (T2) were found significantly superior and were on par in increasing the panicle length. They showed the mean panicle length of 24.86 cm, 24.75 cm, 24.74 cm, 24.55 cm, 24.46 cm, 24.35 cm, 24.30 cm, 24.28 cm, 24.21 cm, 24.20 cm, 24.10 cm, 24.08 cm, 24.09cm and 24 cm respectively. The treatments *viz.*, cow dung extract 2 % (T13), copper hydroxide 0.15% (T20), vermicompost bacteria VB-67 (T5) and streptocycline 250 ppm (T11) were found on par. They showed the mean panicle length of 22.52, 22.48, 22.20 and 22.16 respectively. The treatment copper oxychloride 500 ppm (T19) showed the mean panicle length of 20cm. The absolute control showed the minimum mean panicle length of 17.34.

**4.9.2.2.2. Number of filled grains/panicle**

The effect of different treatments on mean number of filled grains/panicle showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), tetracycline 100 ppm (T8), cow dung extract 2% + *P. fluorescens* 2% (T16), cow dung extract 2%+ vermicompost extract 2% (T15), vermicompost extract 2% + *P. fluorescens* 2% (T17), tetracycline 50 ppm (T7), bacteria from vermicompost VB-69 (T6), streptocycline 250 ppm (T11) and rhizosphere bacteria RR-53 (T2) were found significantly superior in increasing the number of filled grains per panicle. They showed the mean number of filled grains per panicle of 95.44, 90.32, 86.70, 86.04, 85.92, 85.32, 82.84, 82.80 and 82.06 respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), bacteria from cow dung CB-39 (T4), streptocycline 200 ppm (T10), endosphere bacteria RE-1 (T3), Bactrinashak 250 ppm (T12), KAU-Pf1(T20), vermicompost extract 2% (T14) and copper hydroxide 0.15% (T19) were ranked next in increasing the number of filled grains per panicle, 81.56, 80.86, 80.68, 80.64, 80.24, 79.94, 76.88 and 76.42 respectively. The treatment cow dung extract 2 % (T13) showed the mean filled grain number of 76.32. The treatment bacteria from vermicompost VB-67 (T5) showed the mean filled grain number of 69.92. The treatment copper oxychloride 500 ppm (T18) showed the mean filled

grain number of 65.44. The absolute control (T21) showed the mean filled grain number of 59.34.

#### 4.9.2.2.3. Thousand seed weight (g)

The effect of different treatments on mean 1000 seed weight showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), rhizosphere bacteria RR-26 (T1), vermicompost extract 2% + *P. fluorescens* 2% (T17), tetracycline 100 ppm (T8), endosphere bacteria RE-1 (T3), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from vermicompost VB-69 (T6), Bactrinashak 250 ppm (T12), rhizosphere bacteria RR-53 (T2), cow dung extract 2%+ vermicompost extract 2% (T15), streptocycline 250 ppm (T11), KAU-Pf1 (T20), tetracycline 50 ppm (T7), streptocycline 200 ppm (T10) and bacteria from cow dung CB-39 (T4) were found significantly on par. They showed the mean 1000 seed weight of 27.55g, 27.44g, 27.43g, 27.42g, 27.41g, 27.40g, 27.39g, 27.31g, 27.30g, 27.21g, 27.20g, 27.10g, 27.02g, 27.01g and 27 g respectively. The treatments *viz.*, cow dung extract 2 % (T13), and vermicompost extract 2% (T14) were found on par. They showed the mean 1000 seed weight of 26.30g and 26 g respectively. The treatment copper hydroxide 0.15% (T19) showed the mean 1000 seed weight of 24.00 g. The treatments viz., copper oxychloride (T18) and absolute control (T21) were found on par. They showed the mean 1000 seed weight of 21.30g and 21.00 g respectively.

#### 4.9.2.2.4. Yield/pot (g)

The effect of different treatments on pot yield showed that the plants which were treated with tetracycline 250 ppm (T9) recorded the maximum pot yield of 109.23 g and were found significantly different from other treatments. The treatments *viz.*, tetracycline 100 ppm (T8) and tetracycline 50 ppm (T7) were found on par. They showed the mean pot yield of 96.46 g and 94.07 g respectively. Treatments *viz.*, bacteria from vermicompost VB-69 (T6), endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4), rhizosphere bacteria RR-53 (T2), rhizosphere bacteria RR-26 (T1), streptocycline 250 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T16), KAU-Pf1(T20), cow dung extract 2% + vermicompost extract

2% (T15), streptocycline 200 ppm (T10) and Bactrinashak 250 ppm (T12) were found on par. They showed the pot yield of 88.21g, 87.60g, 87.11g, 86.83g, 86.60g, 86.46g, 86.05g, 85.77g, 84.68g, 84.85g and 84.67g respectively. The treatments *viz.*, cow dung extract 2 % (T13) and vermicompost extract 2% (T14) were found on par. They showed the pot yield of 71.79g and 71.39 g respectively. The plants which treated with copper hydroxide (0.15%) recorded the pot yield of 59.72 g. The plants which treated with the treatment bacteria from vermicompost (T5) showed the pot yield of 72.12g. The treatment copper oxychloride 500 ppm (T18) and absolute control (T21) were found on par. They showed the pot yield of 43.13 and 35.55g respectively.

**4.9.2.2.5. Straw yield (g)**

The effect of different treatments on mean fodder yield showed that rhizosphere bacteria RR-26 (T1), tetracycline 250 ppm (T9), vermicompost slurry 2% + *P.fluorescens* 2% (T17), endosphere bacteria RE-1 (T3), rhizosphere bacteria RR-53 (T2), bacteria from cow dung CB-39 (T4), tetracycline 50 ppm (T7), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from vermicompost VB-69 (T6), tetracycline 100 ppm (T8) and KAU-Pf1(T20) were showed significantly higher straw yield and were found on par. They showed the mean straw yield of 88.80g, 87.80g, 87.60g, 87.50g, 87.20g, 87.10g, 87.00g, 86.81g, 86.80g, 86.60g and 86.10g respectively. The treatments *viz.*, Bactrinashak 250 ppm (T12), streptocycline 250 ppm (T11), cow dung extract 2% + vermicompost extract 2% (T15) and bacteria from vermicompost VB-67 (T5) were found on par. They showed the mean straw yield of 80.40g, 79.95g, 79.80g and 79.00g respectively. The treatments *viz.*, streptocycline 200 ppm (T10), cow dung extract 2 % (T13), vermicompost extract 2% (T14) and copper hydroxide 0.15% (T19) were found on par. They showed the mean straw yield of 70.61g, 70.60g, 70.20g, and 69.20g respectively. The treatments *viz.*, copper oxychloride 500 ppm (T19) and absolute control (T21) were found on par. They showed the mean straw yield of 52.50g and 50.40g respectively.

**4.10. Compatibility of bacterial antagonists with agrochemicals against *Xoo***

The compatibility of the six promising antagonistic bacteria *viz.*, RE-1 (rice endosphere bacteria from Kodallur), RR-26 (rice rhizosphere bacteria from Nenmara), RR-53

(rice rhizosphere bacteria from Pattambi) CB-39 (bacteria from cow dung), VB-67 and VB-69 (bacteria from vermicompost) along with reference culture of KAU-(Pf1) with pesticides and fertilizers among themselves in two way combinations were studied and the results are presented in Table 24.

In the table, the inhibition presented as interaction of 1 with 1, 2 with 2, likewise represents, the individual effect of antagonist, pesticide or fertilizer as the case may be. It was considered as 'a' or 'b' as the study dealt with the individual and combined effect of these two materials. Therefore,'a' and 'b' represent the individual effect (inhibition) on the pathogen. Likewise, the value in the interaction of 1 and 2 represents the combined effect of 1 and 2 and is represented as combined effect of 'a+b'. The data indicated that some combined effects were less than the half of additional value of 'a+b' (less than the individual inhibition effects) denoting that they were non compatible. In some cases, the combined effects were equal to the mathematical addition of a+b, indicating their interaction resulted in additive interaction, while some combinations resulted in the inhibition which was more than the additive effect of the component materials, suggesting a synergistic effect by their interaction. Some values showed that they are better than individual inhibition but not up to the level of addition, they are denoted as compatible. Hence, to interpret the data on compatibility and interaction among the study materials, the following calculations were made. The interaction values obtained. are presented in the Table 25.

$$\text{Interaction value} = \frac{\text{Combined effect of (a+b)}}{\text{Addition of individual effect of a \&b}}$$

For easy understanding of the above table, the following notations are given and the table with 'non compatible', 'compatible', 'additive' and 'synergistic' interactions were made. An error component of 0.05 is allowed.

## Table 24.Compatibility of bacterial antagonists with common agrochemicals against *Xoo*

### Inhibition zone on *Xoo* (cm)

| Sl. No. | Bacterial antagonists& agrochemicals | RR-26 | CB-39 | RE-1 | VB-67 | VB-69 | RR-53 | Pf-1 | Chlorpyriphos | Dimethoate | Triazophos | Quinalphos | Dichlorvos | Carbendazim | Mancozeb | Propiconazole | Hexaconazole | Urea | Rajphos | Muriate of potash | Ammonium sulphate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1 | RR-26 | 2.50 | | | | | | | | | | | | | | | | | | | |
| 2 | CB-39 | 3.83 | 1.70 | | | | | | | | | | | | | | | | | | |
| 3 | RE-1 | 1.13 | 3.86 | 3.00 | | | | | | | | | | | | | | | | | |
| 4 | VB-67 | 3.73 | 3.80 | 1.10 | 1.40 | | | | | | | | | | | | | | | | |
| 5 | VB-69 | 3.73 | 2.70 | 3.20 | 2.90 | 1.40 | | | | | | | | | | | | | | | |
| 6 | RR-53 | 3.93 | 2.33 | 1.46 | 1.33 | 1.90 | 1.80 | | | | | | | | | | | | | | |
| 7 | Pf-1 | 1.36 | 1.46 | 3.36 | 1.20 | 2.86 | 1.56 | 2.50 | | | | | | | | | | | | | |
| 8 | Chlorpyriphos | 1.40 | 0.96 | 1.13 | 1.40 | 1.43 | 1.36 | 3.40 | 2.10 | | | | | | | | | | | | |
| 9 | Dimethoate | 1.83 | 1.40 | 4.26 | 1.53 | 1.76 | 3.00 | 2.90 | 2.90 | 2.16 | | | | | | | | | | | |
| 10 | Triazophos | 1.03 | 1.46 | 1.30 | 1.70 | 2.33 | 4.00 | 3.46 | 3.46 | 4.00 | 2.10 | | | | | | | | | | |
| 11 | Quinalphos | 2.66 | 1.06 | 2.86 | 1.13 | 1.20 | 0.90 | 2.96 | 2.96 | 3.83 | 1.66 | 2.03 | | | | | | | | | |
| 12 | Dichlorvos | 3.50 | 1.20 | 1.26 | 1.93 | 0.00 | 1.03 | 2.56 | 2.56 | 1.73 | 1.00 | 4.16 | 2.16 | | | | | | | | |
| 13 | Carbendazim | 2.73 | 1.23 | 1.10 | 1.10 | 1.06 | 2.16 | 3.60 | 3.60 | 1.13 | 1.20 | 1.30 | 4.00 | 0.96 | | | | | | | |
| 14 | Mancozeb | 0.90 | 1.03 | 1.10 | 2.66 | 1.16 | 0.70 | 1.83 | 1.83 | 2.33 | 1.30 | 1.50 | 4.16 | 2.00 | 3.00 | | | | | | |
| 15 | Propiconazole | 2.83 | 1.23 | 4.13 | 2.03 | 1.30 | 1.23 | 1.26 | 1.26 | 3.66 | 0.00 | 3.00 | 4.00 | 4.00 | 3.50 | 2.66 | | | | | |
| 16 | Hexaconazole | 1.46 | 0.86 | 1.53 | 1.63 | 0.00 | 0.00 | 0.86 | 0.86 | 2.23 | 3.16 | 4.16 | 1.83 | 4.00 | 4.00 | 2.83 | 1.33 | | | | |
| 17 | Urea | 2.00 | 1.00 | 1.30 | 1.16 | 1.33 | 1.40 | 3.30 | 3.30 | 4.06 | 0.00 | 3.93 | 3.16 | 4.10 | 3.00 | 2.00 | 3.00 | 1.10 | | | |
| 18 | Rajphos | 3.70 | 1.03 | 1.33 | 1.43 | 1.70 | 4.50 | 2.06 | 2.06 | 2.33 | 2.00 | 1.60 | 2.00 | 3.50 | 3.00 | 3.16 | 3.16 | 3.16 | 0.73 | | |
| 19 | Muriate of potash | 0.00 | 0.90 | 0.00 | 1.16 | 1.43 | 2.16 | 2.83 | 2.83 | 3.73 | 3.66 | 3.56 | 2.00 | 3.33 | 1.20 | 1.33 | 0.86 | 0.76 | 0.90 | 1.00 | |
| 20 | Ammonium sulphate | 3.60 | 0.00 | 1.60 | 1.20 | 1.76 | 1.36 | 1.80 | 1.80 | 3.66 | 2.00 | 1.90 | 1.00 | 3.00 | 2.50 | 2.16 | 2.16 | 0.86 | 1.00 | 0.90 | 0.83 |

## Table 25. Compatibility of bacterial antagonists with common agrochemicals against *Xoo*

### Assumption Table (a+b)

| Sl. No. | Bacterial antagonists& agrochemicals | RR-26 | CB-39 | RE-1 | VB-67 | VB-69 | RR-53 | Pf-1 | Chlorpyriphos | Dimethoate | Triazophos | Quinalphos | Dichlorvos | Carbendazim | Mancozeb | Propiconazole | Hexaconazole | Urea | Rajphos | Muriate of potash | Ammonium sulphate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1 | RR-26 | 2.50 | | | | | | | | | | | | | | | | | | | |
| 2 | CB-39 | 4.20 | 1.70 | | | | | | | | | | | | | | | | | | |
| 3 | RE-1 | 5.50 | 4.70 | 3.00 | | | | | | | | | | | | | | | | | |
| 4 | VB-67 | 3.90 | 3.10 | 4.40 | 1.40 | | | | | | | | | | | | | | | | |
| 5 | VB-69 | 3.90 | 3.10 | 4.40 | 2.80 | 1.40 | | | | | | | | | | | | | | | |
| 6 | RR-53 | 4.30 | 3.50 | 4.80 | 3.20 | 3.20 | 1.80 | | | | | | | | | | | | | | |
| 7 | Pf-1 | 5.00 | 4.20 | 5.50 | 3.90 | 3.90 | 4.30 | 2.50 | | | | | | | | | | | | | |
| 8 | Chlorpyriphos | 4.60 | 3.80 | 5.10 | 3.50 | 3.50 | 3.90 | 4.60 | 2.10 | | | | | | | | | | | | |
| 9 | Dimethoate | 4.66 | 3.86 | 5.16 | 3.56 | 3.56 | 3.96 | 4.66 | 4.26 | 2.16 | | | | | | | | | | | |
| 10 | Triazophos | 4.60 | 3.80 | 5.10 | 3.50 | 3.50 | 3.90 | 4.60 | 4.20 | 4.26 | 2.10 | | | | | | | | | | |
| 11 | Quinalphos | 4.53 | 3.73 | 5.03 | 3.43 | 3.43 | 3.83 | 4.53 | 4.13 | 4.19 | 4.13 | 2.03 | | | | | | | | | |
| 12 | Dichlorvos | 4.66 | 3.86 | 5.16 | 3.56 | 3.56 | 3.96 | 4.66 | 4.26 | 4.32 | 4.26 | 4.19 | 2.16 | | | | | | | | |
| 13 | Carbendazim | 3.46 | 2.66 | 3.96 | 2.36 | 2.36 | 2.76 | 3.46 | 3.06 | 3.12 | 3.06 | 2.99 | 3.12 | 0.96 | | | | | | | |
| 14 | Mancozeb | 5.50 | 4.70 | 6.00 | 4.40 | 4.40 | 4.80 | 5.50 | 5.10 | 5.16 | 5.10 | 5.03 | 5.16 | 3.96 | 3.00 | | | | | | |
| 15 | Propiconazole | 5.16 | 4.36 | 5.66 | 4.06 | 4.06 | 4.46 | 5.16 | 4.76 | 4.82 | 4.76 | 4.69 | 4.82 | 3.62 | 5.66 | 2.66 | | | | | |
| 16 | Hexaconazole | 3.83 | 3.03 | 4.33 | 2.73 | 2.73 | 3.13 | 3.83 | 3.43 | 3.49 | 3.43 | 3.36 | 3.49 | 2.29 | 4.33 | 3.99 | 1.33 | | | | |
| 17 | Urea | 3.60 | 2.80 | 4.10 | 2.50 | 2.50 | 2.90 | 3.60 | 3.20 | 3.26 | 3.20 | 3.13 | 3.26 | 2.06 | 4.10 | 3.76 | 2.43 | 1.10 | | | |
| 18 | Rajphos | 3.23 | 2.43 | 3.73 | 2.13 | 2.13 | 2.53 | 3.23 | 2.83 | 2.89 | 2.83 | 2.76 | 2.89 | 1.69 | 3.73 | 3.39 | 2.06 | 1.83 | 0.73 | | |
| 19 | Muriate of potash | 3.50 | 2.70 | 4.00 | 2.40 | 2.40 | 2.80 | 3.50 | 3.10 | 3.16 | 3.10 | 3.03 | 3.16 | 1.96 | 4.00 | 3.66 | 2.33 | 2.10 | 1.73 | 1.00 | |
| 20 | Ammonium sulphate | 3.33 | 2.53 | 3.83 | 2.23 | 2.23 | 2.63 | 3.33 | 2.93 | 2.99 | 2.93 | 2.86 | 2.99 | 1.79 | 3.83 | 3.49 | 2.16 | 1.93 | 1.56 | 1.83 | 0.83 |

| Sl.No | Notation | Description | Interaction value |
|---|---|---|---|
| 1 | NC | Non compatible | <0.5 |
| 2 | C | Compatible | 0.5to 1.0 |
| 3 | A | Additive | 1.0 |
| 4 | S | Synergistic | >1.0 |

The compatibility of bacterial antagonists with agrochemicals against *Xoo* was studied and the results are presented in Table 26. The interaction effect revealed that the antagonistic bacteria RR-26 (1), showed additive effect with the antagonistic bacteria VB-67(4) and VB-69 (5) and synergistic effect with the other antagonistic bacteria *viz.*, CB-39(2), RE-1(3), RR-53(6) (Plate 13) and Pf1(7) in inhibiting the *Xoo*. It showed synergistic effect with all the nine pesticides *viz.*, chlorpyriphos (8), dimethoate (9), triazophos (10), quinalphos (11), dichlorvos (12), carbendazim (13), mancozeb (14), propiconazole (15) and hexaconazole (16) in inhibiting the *Xoo*. It showed synergistic effect with urea (17) and non compatible effect with rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

Similarly, the antagonistic bacteria CB-39 (2), showed synergistic effect to the other antagonistic bacteria *viz.*, RE-1 (3), VB-69 (5), RR-53 (6) (Plate 14) and Pf1 (7), but showed non compatible effect with the VB-67 (4) in inhibiting the *Xoo* (Plate 14). It showed synergistic effect with all the nine pesticides *viz.*, carbendazim (13), mancozeb (14), propiconazole (15), hexaconazole (16), chlorpyriphos (8), dimethoate (9), dichlorvos, (12), triazophos (10) and quinalphos (11) in inhibiting the *Xoo*. It showed synergistic effect with the fertilizers *viz.*, urea (17), rajphos (18) and muriate of potash (19) and non compatible effect with ammonium sulphate (20) in inhibiting the *Xoo*.

The antagonistic bacteria RE-1(3), showed synergistic effect with other antagonistic bacteria *viz.*, VB-67 (4),VB-69 (5), RR-53 (6) and Pf1 (7) and with eight pesticides *viz.*, carbendazim (13), mancozeb (14), propiconazole (15), hexaconazole (16), chlorpyriphos (8), dichlorvos (12), triazophos (10) and quinalphos (11) in inhibiting the *Xoo*., but showed non compatible effect with dimethoate in inhibiting the *Xoo*. It showed synergistic effect with urea (17), rajphos (18) and ammonium sulphate (20) but non compatible effect with muriate of potash (19) in inhibiting the *Xoo*.

**Plate 13.Compatibility of antagonistic bacteria**

**(RR-26 + VB-67 & RR-26+RR-53)**

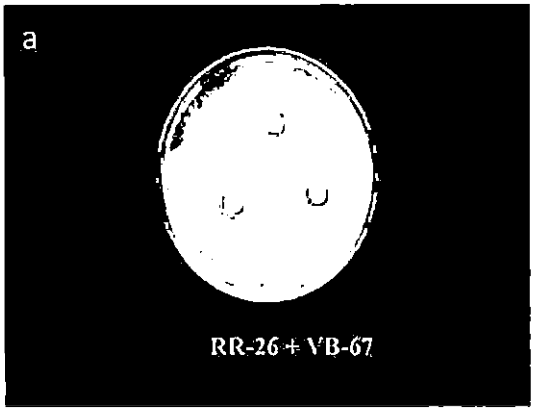
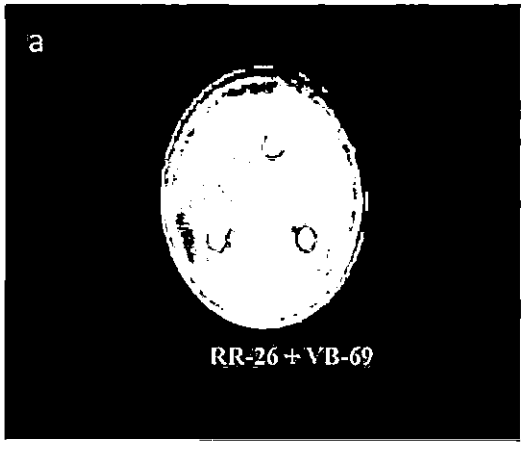




**Plate 14.Compatibility of antagonistic bacteria**

**(RR-26 + VB-69 & CB-39 + VB-69)**



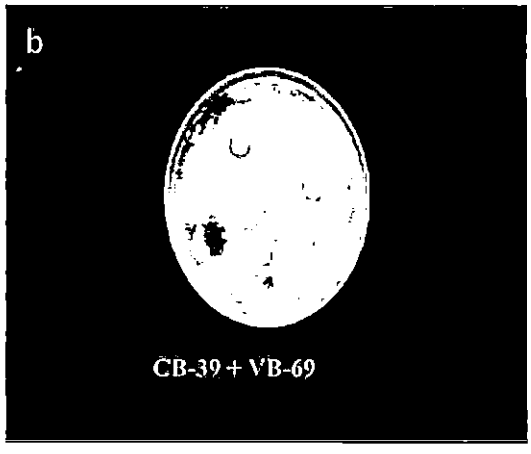



**Table.26.Compatibility of bacterial antagonists with common agrochemicals against *Xoo***

**(Interpretation Table)**

| Sl. No. | Bacterial antagonists& agrochemicals | RR-26 | CB-39 | RE-1 | VB-67 | VB-69 | RR-53 | Pf-1 | Chlorpyriphos | Dimethoate | Triazophos | Quinalphos | Dichlorvos | Carbendazim | Mancozeb | Propiconazole | Hexacnazole | Urea | Rajphos | Muriate of potash | Ammonium sulphate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1 | RR-26 | C | | | | | | | | | | | | | | | | | | | |
| 2 | CB-39 | S | C | | | | | | | | | | | | | | | | | | |
| 3 | RE-1 | S | S | C | | | | | | | | | | | | | | | | | |
| 4 | VB-67 | A | NC | S | C | | | | | | | | | | | | | | | | |
| 5 | VB-69 | A | S | S | NC | C | | | | | | | | | | | | | | | |
| 6 | RR-53 | S | S | S | S | S | C | | | | | | | | | | | | | | |
| 7 | Pf-1 | S | S | S | S | S | S | C | | | | | | | | | | | | | |
| 8 | Chlorpyriphos | S | S | S | S | S | S | S | C | | | | | | | | | | | | |
| 9 | Dimethoate | S | S | NC | S | S | S | S | S | C | | | | | | | | | | | |
| 10 | Triazophos | S | S | S | S | S | NC | S | S | S | C | | | | | | | | | | |
| 11 | Quinalphos | S | S | S | S | S | S | S | S | S | S | C | | | | | | | | | |
| 12 | Dichlorvos | S | S | S | S | NC | S | S | S | S | S | C | C | | | | | | | | |
| 13 | Carbendazim | S | S | S | S | S | S | NC | NC | S | S | S | NC | C | | | | | | | |
| 14 | Mancozeb | S | S | S | S | S | S | S | S | S | S | S | S | S | C | | | | | | |
| 15 | Propiconazole | S | S | S | S | S | S | S | S | S | NC | S | S | NC | S | C | | | | | |
| 16 | Hexaconazole | S | S | S | S | NC | NC | S | S | S | S | NC | S | NC | S | S | C | | | | |
| 17 | Urea | S | S | S | S | S | S | S | NC | S | NC | NC | C | NC | S | S | NC | C | | | |
| 18 | Rajphos | NC | S | S | S | S | NC | S | S | S | S | S | S | NC | S | S | NC | NC | C | | |
| 19 | Muriate of potash | NC | S | NC | S | S | S | S | S | NC | NC | NC | S | NC | S | S | S | S | S | C | |
| 20 | Ammonium sulphate | NC | NC | S | S | S | S | S | S | NC | S | S | S | NC | S | S | C | S | S | S | C |

NC- Non compatible C- Compatible S – Synergistic

The antagonistic bacteria VB-67 (4) showed synergistic effect with the antagonistic bacteria *viz.*, RR-53 (6) and Pfl (7) but showed non compatible effect with VB-69 (5) in inhibiting the *Xoo*. It showed synergistic effect with all the nine pesticides namely carbendazim (13), mancozeb (14), propiconazole (15), hexaconazole (16), chlorpyriphos (8), dimethoate (9), dichlorvos (12), triazophos (10) and quinalphos (11) and four fertilizers *viz.*,urea (17), rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

The antagonistic bacteria VB-69 (5), showed synergestic effect with the other bacteria *viz.*, RR-53 (6) and Pf-1(7) in inhibiting the *Xoo*. It showed synergistic effect with seven pesticides *viz.*, carbendazim (13), mancozeb (14), propiconazole (15), chlorpyriphos (8), dimethoate (9), triazophos (10) and quinalphos (11) but showed non compatible effect with dichlorvos (12) and hexaconazole (16) in inhibiting *Xoo*. It showed synergistic effect with the four fertilizers *viz.*, urea (17), rajphos (18), muriate of potash (19) and ammonium sulphate (20) used in the study in inhibiting the *Xoo*.

The antagonistic bacteria RR-53 (6), showed synergistic effect with seven pesticides *viz.*, carbendazim (13), mancozeb (14), propiconazole (15), chlorpyriphos (8), dimethoate (9), dichlorvos (12) and quinalphos (11) but showed non compatible effect with triazophos (10) and hexaconazole (16) in inhibiting the *Xoo*. It showed synergistic effect with the fertilizers *viz.*, urea (17), muriate of potash (19) and ammonium sulphate (20) and non compatible effect with rajphos (18) in inhibiting the *Xoo*.

The KAU-Pfl (7) showed synergistic effect with eight pesticides *viz.*, mancozeb (14), propiconazole (15), hexaconazole (16), chlorpyriphos (8), dimethoate (9), dichlorvos (12), triazophos (10) and quinalphos (11) but showed non compatible effect with carbendazim (13) in inhibiting the *Xoo*. It showed synergistic effect with the fertilizers *viz.*, rajphos (18), muriate of potash (19) and ammonium sulphate (20), but showed non compatible effect with urea in inhibiting the *Xoo*.

The insecticide chlorpyriphos (8) showed synergistic effect with seven pesticides *viz.*, mancozeb (14), propiconazole (15), hexaconazole (16), dimethoate (9), dichlorvos (12),

triazophos (10) and quinalphos (11) but showed non compatible effect with carbendazim (13) in inhibiting the *Xoo*. It showed synergistic effect with the fertilizers *viz*., rajphos (18), muriate of potash (19) and ammonium sulphate (20), but showed non compatible effect with urea (17) in inhibiting *Xoo*.

The insecticide dimethoate (9) showed synergistic effect with the seven pesticides *viz*., carbendazim (13), mancozeb (14), propiconazole (15), hexaconazole (16), dichlorvos (12), triazophos (10) and quinalphos (11) in inhibiting *Xoo*. It showed synergistic effect with to the fertilizers *viz*., urea (17) and rajphos (18) and non compatible effect with muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

The insecticide triazophos (10) showed synergistic effect with the five pesticides *viz*., carbendazim (13), mancozeb (14), hexaconazole (16), dichlorvos (12) and quinalphos (11) and non compatible effect with propiconazole (15) in inhibiting *Xoo*. It showed synergistic effect with the fertilizers *viz*., rajphos (18) and ammonium sulphate (20) and non compatible effect with urea (17) and muriate of potash (19) in inhibiting the *Xoo*.

The insecticide quinolphos (11) showed compatible effect with the insecticide dichlorvos (12) and showed synergistic effect with the fungicides, carbendazim (13), mancozeb (14) and propiconazole (15) but showed non compatible effect with hexaconazole (16) in inhibiting *Xoo*. It showed synergistic effect with the fertilizers rajphos (18) and ammonium sulphate (20) and non compatible effect with urea (17) and muriate of potash (20) in inhibiting the *Xoo*.

The insecticide dichlorvos (12) showed synergistic effect with the fungicides mancozeb (14), propiconazole (15) and hexaconazole (16), but showed non compatible effect with the fungicide, carbendazim (13) in inhibiting *Xoo*. It showed compatible effect with urea (17) and showed synergistic effect with rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting *Xoo*.

The fungicide carbendazim (13) showed synergistic effect with the fungicide mancozeb (14) and showed non compatible effect with the fungicides propiconazole (15) and hexaconazole (16) in inhibiting the *Xoo*. It showed non compatible effect with the four fertilizers *viz*., urea (17), rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

The fungicide mancozeb (14) showed synergistic effect with propiconazole (15) and hexaconazole (16) in inhibiting the *Xoo*. It showed synergistic effect with the four fertilizers *viz*.,urea (17), rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

The fungicide propiconazole (15) showed synergistic effect with the fungicide hexaconazole (16) and showed synergistic effect with the four fertilizers *viz*., urea (17), rajphos (18), muriate of potash (19) and ammonium sulphate (20) in inhibiting the *Xoo*.

The fungicide hexaconazole (16) showed non compatible effect with the fertilizers urea (17) and rajphos (18), synergistic effect with muriate of potash (19) and compatible effect with ammonium sulphate (20) in inhibiting the *Xoo*.

The fertilizer urea (17) showed non compatible effect with the fertilizer rajphos (18), but it showed synergistic effect with the fertilizers muriate of potash (19) and ammonium sulphate (20) in inhibiting *Xoo*.

The fertilizer rajphos (18) showed synergistic effect with the fertilizers muriate of potash (19) and ammonium sulphate (20) in inhibiting *Xoo*.

The fertilizer muriate of potash (19) showed synergistic effect with the fertilizer ammonium sulphate (20) in inhibiting the *Xoo*.

## 4.12. Mode of action of promising bacterial isolates

In the preliminary screening of six bacterial isolates including the reference culture of *P. fluorescens* (Pf1) from the pot culture experiment, six bacterial isolates which showed a promising effect in increasing the yield and yield attributing characters were selected and studied further for their growth promoting characteristics as given below.

### 4.12.1. Production of hydrogen cyanide

All the six selected bacterial isolates along with Pf-1 were tested for their ability to produce hydrogen cyanide (HCN). It was observed that all the isolates were cyanogenic in nature and therefore scored as 4, where as Pf1 was not cyanogenic and scored as 1 (Table 27) (Plate 15).

### 4.12.2. Production of ammonia

Production of ammonia by the bacterial isolates was detected by change in colour in the peptone broth media on addition of Nessler's reagent. Different isolates produced varying levels of ammonia (Table 28) (Fig 5). All the six isolates and Pf1 showed more production of ammonia and were thus scored as 4.

### 4.12.3. Phosphorous (P) solubilization

The phosphorous solubilization capacity of the selected bacterial isolates was tested in Pikovaskya's TCP agar as well as in its broth. Of the six bacterial isolates and reference cultures of KAU- (Pf1) showed phosphate solubilization zones on Pikovaskya's TCP medium plates (Table.29) (Fig 6). The zone of 'P' solubilization on agar plates ranged from 3.5 to 6 mm within one week. From the table, it is clear that RR-26 showed the maximum diameter of clear zone of 6 mm followed by CB-39 (5.87).The isolate RE-1, VB-69 and the Pf1 produced zone of 5.5 mm (Plate 16). Broth assay also proved the efficacy of the isolates in solubilization of phosphorous which ranged from 2.67 to 5 mg/50 ml of the culture media, the maximum being with RE-1, VB-69 and Pf1.

**Plate 15. Production of hydrogen cyanide by promising antagonists**

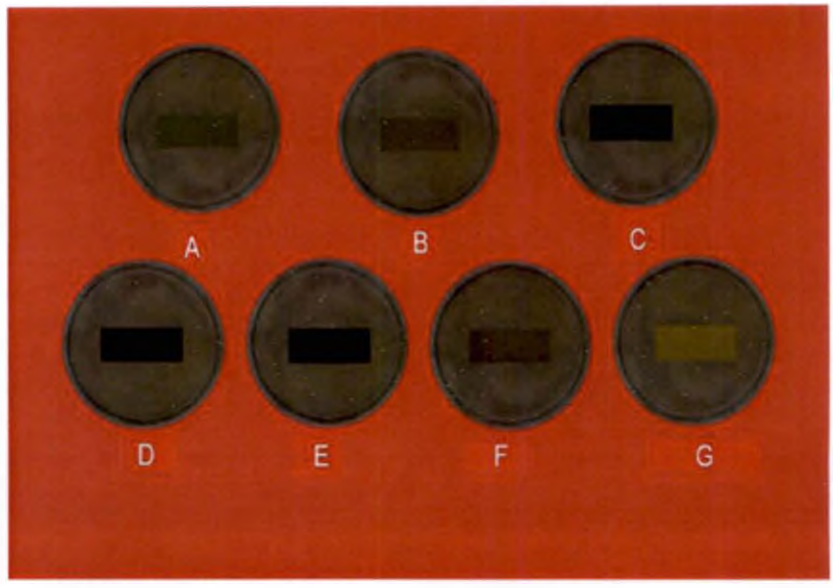
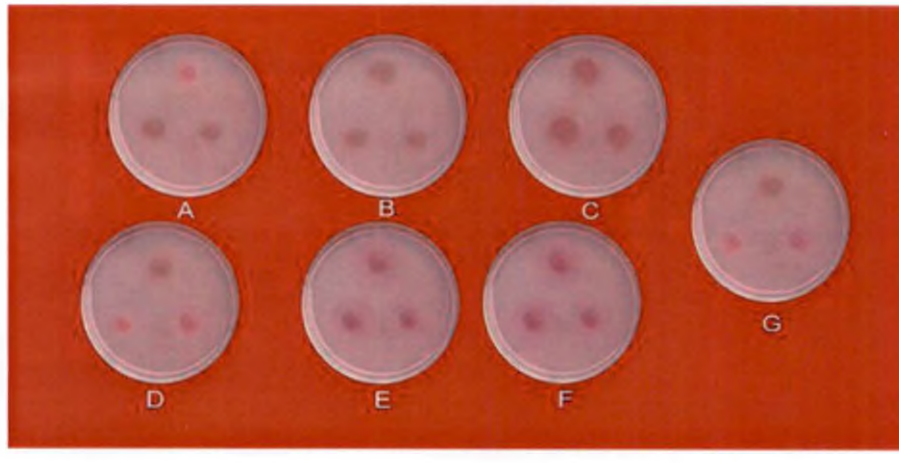



**Plate 16. Phosphorus solubilization by promising antagonists**




A : Rice endosphere bacteria (RE-1)
B : Rice rhizosphere bacteria (RR-26)
C : Rice rhizosphere bacteria (RR-53)
D : Cow dung bacteria (CB-39)
E : Vermicompost bacteria (VB-67)
F : Vermicompost bacteria (VB-69)
G : *Pseudomonas fluorescens*(reference culture)

**Table 27. Production of hydrogen cyanide by promising bacterial isolates**

| Sl.No. | *Isolates | Score |
|---|---|---|
| 1 | RE-1 | 4 |
| 2 | RR-26 | 4 |
| 3 | RR-53 | 4 |
| 4 | CB-39 | 4 |
| 5 | VB-67 | 4 |
| 6 | VB-69 | 4 |
| 7 | Pf-1 | 1 |
| 8 | Control | 1 |

Score chart: Nil: 1, Low: 2, Medium: 3, High: 4

*RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Table 28. Production of ammonia by promising bacterial isolates**

| Sl.No. | *Isolates | Score |
|---|---|---|
| 1 | RE-1 | 4 |
| 2 | RR-26 | 4 |
| 3 | RR-53 | 4 |
| 4 | CB-39 | 4 |
| 5 | VB-67 | 4 |
| 6 | VB-69 | 4 |
| 7 | Pf-1 | 4 |
| 8 | Control | 1 |

Score chart: Nil: 1, Low: 2, Medium: 3, High: 4

*RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Table 29. Phosphorus solubilization by promising bacterial isolates**

| Sl.No. | Isolate | *P solubilization Zone (mm) | P solubilization (mg $ml^{-1}$) | Score |
|---|---|---|---|---|
| 1 | RE-1 | 5.50 | 5.00 | 2 |
| 2 | RR-26 | 6.00 | 3.00 | 2 |
| 3 | RR-53 | 3.15 | 2.67 | 1 |
| 4 | CB-39 | 5.87 | 5.45 | 2 |
| 5 | VB-67 | 3.92 | 4.57 | 2 |
| 6 | VB-69 | 5.50 | 5.00 | 2 |
| 7 | Pf-1 | 5.50 | 5.00 | 2 |
| 8 | Control | 0 | 0 | 0 |

*Mean of three replications

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Table 30. Production of indole acetic acid (IAA) by antagonistic bacterial isolates**

| Sl.No. | Isolate | *IAA (µg $ml^{-1}$) | Score |
|---|---|---|---|
| 1 | RE-1 | 18.01 | 2 |
| 2 | RR-26 | 19.02 | 2 |
| 3 | RR-53 | 15.02 | 2 |
| 4 | CB-39 | 20.32 | 2 |
| 5 | VB-67 | 16.02 | 2 |
| 6 | VB-69 | 19.05 | 2 |
| 7 | Pf-1 | 18.50 | 2 |
| 8 | Control | 0 | 1 |

*Mean of three replications

Score: > 0<15 µg $ml^{-1}$ = 1; > 16<30 µg $ml^{-1}$=2; > 31<45 µg $ml^{-1}$=3; > 46 µg $ml^{-1}$= 4

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Fig 5. Production of ammonia by promising bacterial isolates**

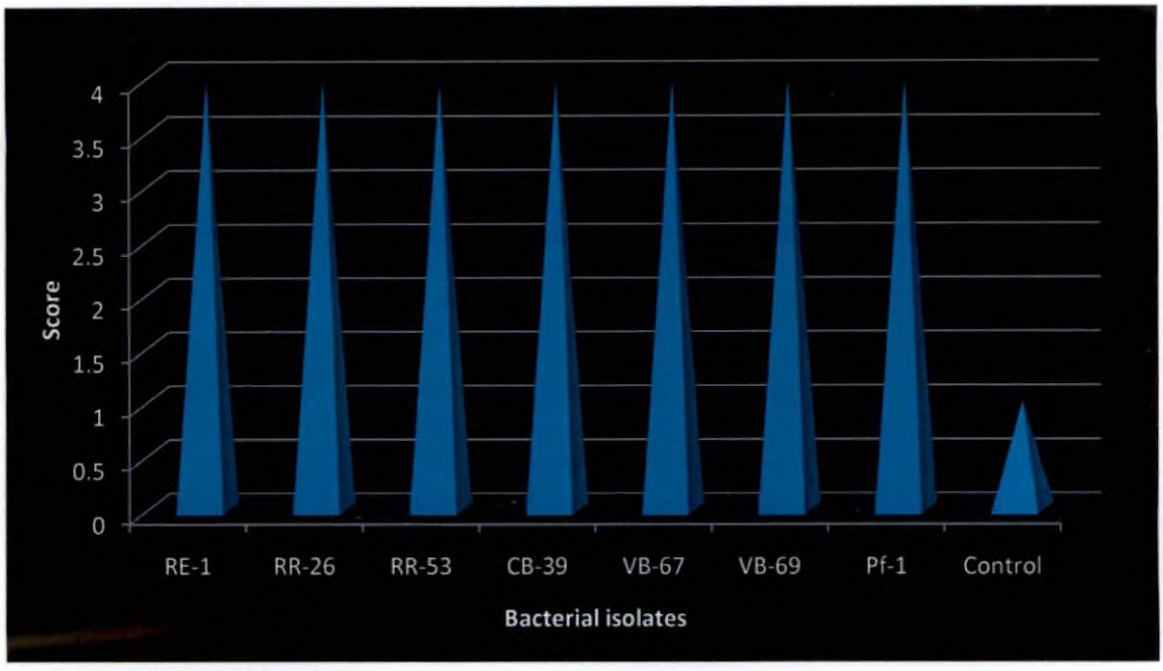
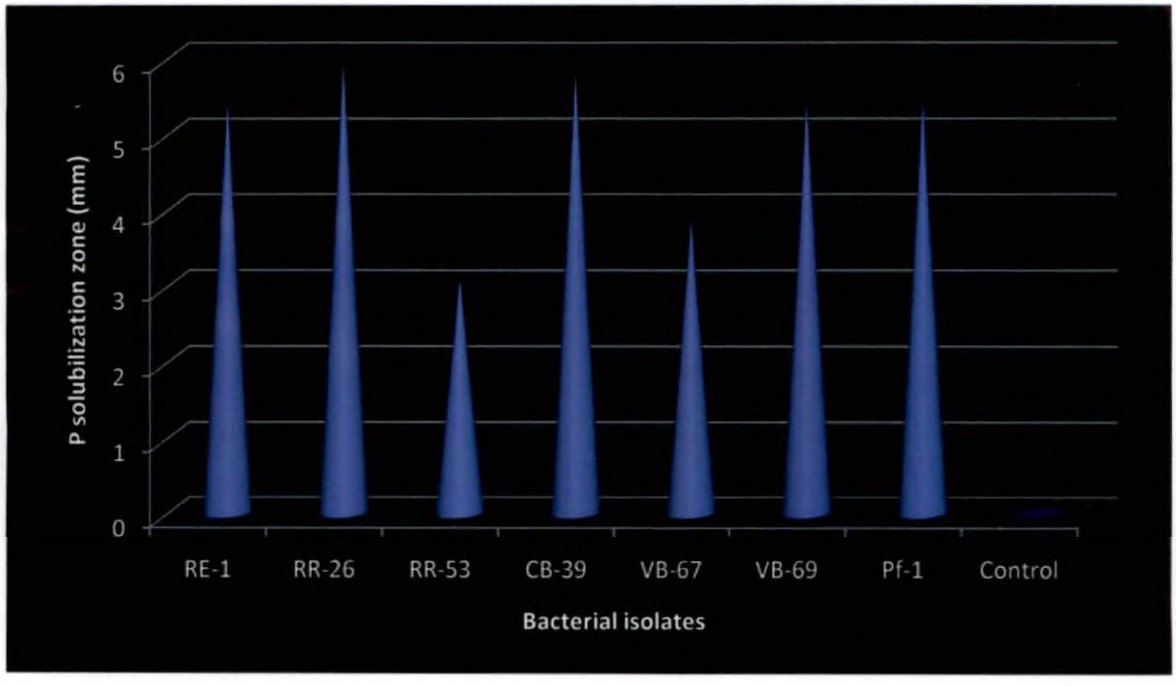



**Fig 6. Phosphorus solubilization by promising bacterial isolates**



### 4.12.4. Assay of growth promoting hormone-Indole Acetic Acid (IAA)

All the isolates produced varying levels of IAA ranging from 15.02 to 20.32 $\mu gml^{-1}$(Table 30)(Fig 7). The maximum amount of IAA was produced by CB-39. The remaining isolates produced comparatively high quantity of IAA which ranged from 15.02 to 20.32 $\mu gml^{-1}$ and were therefore scored as 2.

### 4.12.5. Detection of siderophores

The ability of the six promising bacterial isolates including one reference culture Pf1 was tested for their capacity to produce siderophores.

#### 4.12.5.1. Detection of siderophores by UV fluorescence method

Among the bacterial cultures tested, all the isolates *viz.,* RE-1, RR-26, RR-53, CB - 39, VB-67 and VB-69 and the reference culture Pf1 showed fluorescence under U.V. light (Plate 17).

#### 4.12.5.2. Chrome azurol (CAS) assay

The data presented in Table.31, indicated the production of siderophores by all bacterial strains. VB-69 produced a zone of colouration of 26 mm. The other cultures produced a zone of colouration from 15 to 25 mm.

### 4.12.6. Production of non volatile metabolites

Production of non volatile metabolites by the promising antagonists in comparison with refernce culture Pf1 was tested by the cellophane method as described in materials and methods. The results of this experiment are furnished in Table.32. At three days after inoculation, the per cent inhibition of the pathogen varied from 25 to 50. The maximum inhibition was recorded by RE-1 (50) and VB-69 (50) followed by RR-26 (45) and Pf1

**Fig 7. Production of indole acetic acid (IAA) by promising bacterial isolates**

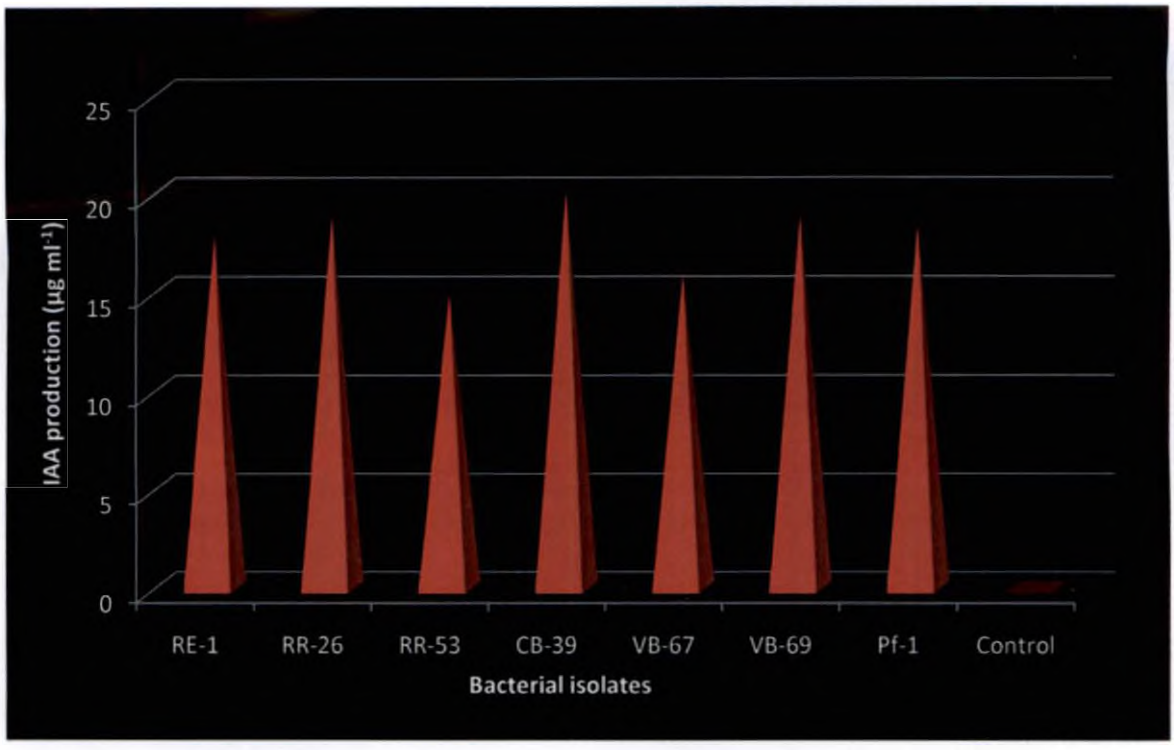
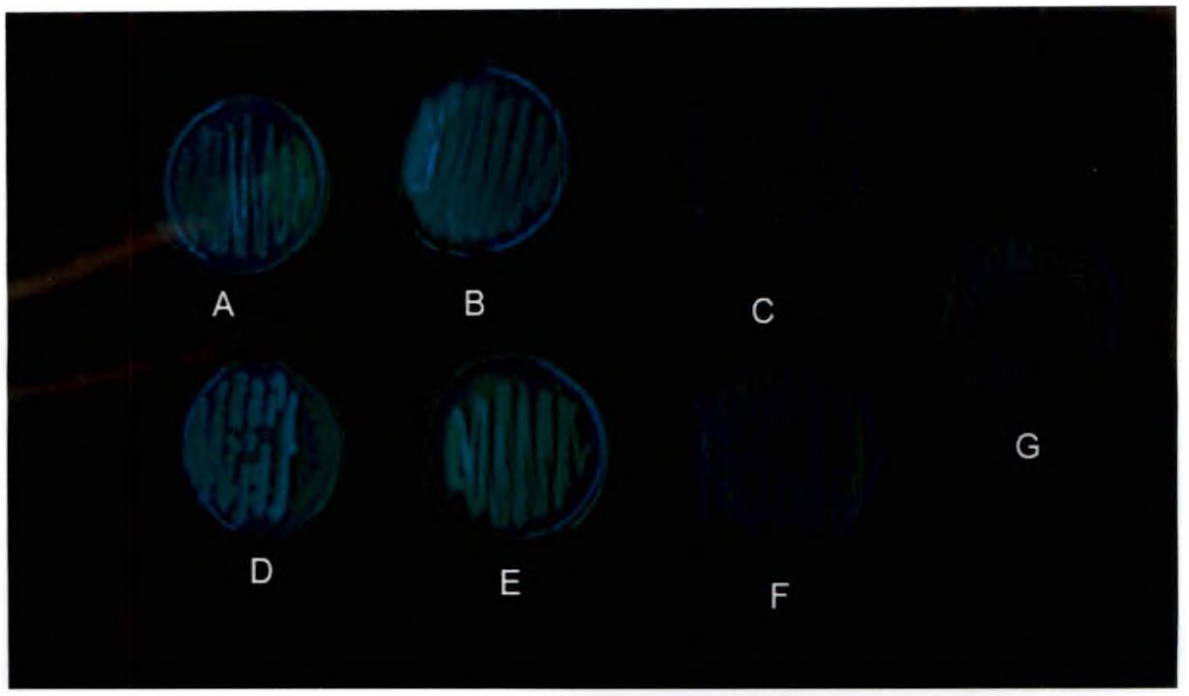


**Plate 17. Detection of siderophores by U.V fluorescence**




A : Rice endosphere bacteria (RE-1)
B : Rice rhizosphere bacteria (RR-26)
C : Rice rhizosphere bacteria (RR-53)
D : Cow dung bacteria (CB-39)
E : Vermicompost bacteria (VB-67)
F : Vermicompost bacteria (VB-69)
G : *Pseudomonas fluorescens*(reference culture)

(45).The minimum per cent inhibition was recorded in RR-53 (25), CB-39 (25) and VB - 67(25).

### 4.13. Field experiment for the management of bacterial blight disease of rice

A field experiment was laid out to study the antagonistic and growth promoting efficiency of five selected bacterial antagonists, two bacterial consortium, one standard bacterial biocontrol agent (Pf1) along with one chemical, three antibiotics and two organics against the highly virulent Polpully isolate (XPLY-5) of *Xoo* (Plate 18). Observations on per cent disease incidence, per cent disease severity and co-efficient of infection were recorded at 60 DAT. The results are presented in the Table 33.

#### 4.13.1. Disease reaction due to various treatments

The data revealed that the per cent disease incidence of various treatments varied from 43.33 to 83.33. All the treatments were found significantly effective in managing the disease than the absolute control. Among the various treatments, the plots which received tetracycline 100 ppm (T12) ranked first with PDI value of 43.33 followed by tetracycline 50 ppm (T11-46.66) and were found to be highly effective in managing the disease. The plots which received endosphere bacteria RE-1(T3), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T5), bacterial consortium (RE-1+CB-39), bacterial consortium (CB-39+VB-69) and streptocycline 250 ppm (T9) ranked third with PDI value of 50. The plots treated with Bactrinashak 250 ppm (T10) showed the PDI value of 53.33. All the above nine treatments were found on par with each other in checking the disease incidence. The treatments *viz*., rhizosphere bacteria RR-26 (T1), cow dung extract 2% + vermicompost extract 2% (T14), cow dung extract 2% + KAU-(Pf1) 2% (T15) and KAU-(Pf1) 2% (T16), rhizosphere bacteria (RR-53), streptocycline 50 ppm (T8) and cow dung extract (2%) (T13) were found on par in managing the disease. They recorded the PDI values of 56.66 except rhizosphere bacteria (RR-53), streptocycline 50 ppm (T8) and cow dung extract (2%) (T13). They showed the PDI value of 60, 63.33 and 66.66 respectively. The absolute control (T17) recorded the maximum PDI value of 83.33.

**Table 31. Siderophore production by promising bacterial isolates by CAS assay**

| Sl.No. | Isolates | Zone of colouration on CAS plates (mm)* |
|---|---|---|
| 1 | RE-1 | 22 |
| 2 | RR-26 | 13 |
| 3 | RR-53 | 25 |
| 4 | CB-39 | 15 |
| 5 | VB-67 | 20 |
| 6 | VB-69 | 26 |
| 7 | Pf-1 | 22 |
| 8 | Control | 0 |

*Mean of three replications

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Table 32. Inhibitory effect of *Xoo* by diffusible non-volatile metabolites**

| Sl.No. | Isolates | 3 DAI | |
|---|---|---|---|
| | | Inhibition (mm) | PIOC* |
| 1. | RR-26 | 0.90 | 45.00 |
| 2. | RR-53 | 0.50 | 25.00 |
| 3. | RE-1 | 1.00 | 50.00 |
| 4. | CB-39 | 0.50 | 25.00 |
| 5. | VB-67 | 0.50 | 25.00 |
| 6. | VB-69 | 1.00 | 50.00 |
| 7. | Pf-1 | 0.90 | 45.00 |

*Per cent inhibition over control

RE- Rice endosphere bacteria
RR- Rice rhizosphere bacteria
CB- Cow dung bacteria
VB- Vermicompost bacteria
Pf1 – *Pseudomonas fluorescens*

**Plate 18.View of the field experiment**


**Plate 19. Field view of promising treatments (T1 & T4)**


**Table 33. Evaluation of antagonists, organics and agrochemicals for the management of bacterial blight disease**

| Treatments | Disease reaction at 60 DAT | | |
|---|---|---|---|
| | PDI | PDS | CI |
| T1. Rhizosphere bacteria (RR-26) | 56.66 | 19.19 | 10.86 |
| T2. Rhizosphere bacteria (RR-53) | 60.00 | 23.52 | 14.11 |
| T3. Endosphere bacteria (RE-1) | 50.00 | 18.72 | 9.36 |
| T4. Cow dung bacteria (CB-39) | 50.00 | 18.54 | 9.29 |
| T5. Vermicompost bacteria (VB-69) | 50.00 | 18.73 | 9.37 |
| T6. Bacterial consortium (RE-1 + CB-39) | 50.00 | 17.83 | 8.91 |
| T7. Bacterial consortium (CB-39+VB-69) | 50.00 | 18.42 | 9.21 |
| T8. Streptocycline spray (50 ppm) as per POP | 63.33 | 30.38 | 19.24 |
| T9. Streptocycline 250 ppm | 50.00 | 19.69 | 9.85 |
| T10. Bactrinashak 250 ppm | 53.33 | 19.71 | 10.46 |
| T11. Tetracycline 50 ppm | 46.66 | 17.11 | 7.98 |
| T12. Tetracycline 100 ppm | 43.33 | 15.08 | 6.52 |
| T13. Cow dung slurry spray (2%) as per POP | 66.66 | 30.84 | 20.52 |
| T14. Cow dung slurry 2% +Vermicompost slurry 2% | 56.66 | 21.68 | 12.30 |
| T15. Cow dung slurry 2% + KAU- (Pf1) 2% | 56.66 | 18.63 | 10.52 |
| T16. KAU- (Pf1) 2% | 56.66 | 21.39 | 11.91 |
| T17. Absolute control | 83.33 | 49.32 | 41.11 |
| CD at 5% | 10.59 | 1.21 | 2.33 |

The per cent disease severity (PDS) varied from 15.08 to 49.32. All the treatments were found significantly effective in managing the disease than the absolute control. Among the various treatments, the plots which received tetracycline 100 ppm (T12) ranked first with PDS value of 15.08 followed by tetracycline 50 ppm (T11- 17.11) and bacterial consortium (RE-1 + CB-39) (T6-17.83) were found to be significantly effective in managing the disease. The treatments *viz*., bacterial consortium (T7- CB-39+VB-69), bacteria from cow dung (T4-CB-39), cow dung extract 2% + KAU-(Pf1) 2% (T15), endosphere bacteria (T3-RE-1), bacteria from vermicompost (T5-VB-69) and rhizosphere bacteria (T1-RR-26) ranked next in managing the disease with the PDS values of 18.42, 18.54, 18.63, 18.72, 18.73 and 19.19 respectively.The plots which received streptocycline 250 ppm (T9) and Bactrinashak 250 ppm (T10) were found on par in managing the disease with the PDS value of 19.69 and 19.71 respectively. KAU-(Pf1) 2% (T16) recorded the PDS value of 21.39 followed by cow dung extract 2% + vermicompost extract 2% (T14) with the PDS value of 21.68. Rhizosphere bacteria RR-53 (T2), streptocycline 50 ppm (T8) and cow dung extract 2% (T13) showed the PDS values of 23.52, 30.38 and 30.84 respectively. The absolute control (T17) recorded the maximum PDS value of 49.32.

The coefficient of infection value among the treatments varied from 6.52 to 41.11. All the treatments were found to be significantly effective in managing the disease than the absolute control (Fig 8). Among the various treatments, tetracycline 100 ppm (T12) and tetracycline 50 ppm (T11) were found significantly superior in managing the disease with the CI values of 6.52 and 7.98 respectively. The treatments *viz*., bacterial consortium RE-1+CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1 (T3), bacteria from vermicompost VB-69 (T5), streptocycline 250 ppm (T9), Bactrinashak 250 ppm (T10), cow dung extract 2% + KAU-(Pf1) 2% (T15), rhizosphere bacteria RR-26 (T1) and KAU-(Pf1) 2% (T16) were stood next in managing the disease with the CI values of 8.91, 9.21, 9.29, 9.36, 9.37, 9.85, 10.46, 10.52, 10.86 and 11.91 respectively (Plate 19). The treatments *viz*., cow dung extract 2% + vermicompost extract 2% (T14) and rhizosphere bacteria RR-53 (T2) were found on par in managing the disease with the CI values of 12.30 and 14.11 respectively. The treatments *viz*., streptocycline 50 ppm (T8) and

cow dung extract 2% (T13) were found on par with the CI values of 19.24 and 20.52 respectively. The absolute control (T17) recorded the maximum CI value of 41.11.

### 4.13.2. Effect of different treatments on biometric characters

The effect of different treatments on biometric characters of the plants was studied. The results are presented in the Table 34.

#### 4.13.2.1. Growth characters

#### 4.13.2.1.2. Plant height (cm)

The effect of different treatments on plant height was studied at 60 DAT. The treatments *viz*., bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1(T3), rhizosphere bacteria RR-26 (T1) and bacteria from vermicompost VB-69 (T5) were found superior in increasing the plant height (Fig 9). They showed the mean plant height of 86.33 cm, 86.16 cm, 85.73cm, 84.93 cm, 84.06 cm and 83.60 cm respectively. The treatments *viz*., cow dung extract 2% + KAU-(Pf1) 2% (T15), KAU-(Pf1) 2% (T16), cow dung extract 2% + vermicompost extract 2% (T14) and Bactrinashak 250 ppm (T10) were found next in increasing the plant height. They showed the mean plant length of 82.40cm, 82.06 cm, 81.73cm and 81.36 cm respectively. The treatments *viz*., streptocycline 250 ppm (T9), tetracycline 100 ppm (T12), rhizosphere bacteria RR-53 (T2) and tetracycline 50 ppm (T11) were found on par with the mean plant height of 79.60 cm, 79.51 cm, 79.43 and 79.10 cm respectively. The treatments *viz*., streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par with the mean shoot length of 75.70 cm and 75.60 cm respectively. The absolute control (T17) recorded the mean shoot length of 72.93cm.

#### 4.13.2.1.3. Number of productive tillers

The effect of different treatments on number of productive tillers was studied at 60 DAT. The treatments *viz*., tetracycline 100 ppm (T12), tetracycline 50 ppm (T11) and bacterial

**Table 34. Effect of antagonists, organics and agrochemicals on biometric characters under field condition**

| Treatments | Plant height (cm) | No. of productive tillers | Root length (cm) | Root wt.(g) | Panicle length (cm) | No.of filled grains/ panicle | 1000 seed weight (g) | Yield (kg/ha) | Straw yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| T1 | 84.06 | 10.43 | 18.90 | 15.50 | 19.50 | 94.00 | 26.8 | 2749.90 | 3314.28 |
| T2 | 79.43 | 8.80 | 17.90 | 14.00 | 18.13 | 88.66 | 25.2 | 2557.03 | 2814.30 |
| T3 | 84.93 | 10.53 | 19.00 | 15.00 | 20.16 | 90.66 | 27.6 | 2857.04 | 3435.28 |
| T4 | 85.73 | 10.83 | 19.95 | 14.90 | 20.50 | 92.33 | 27.8 | 2899.89 | 3487.76 |
| T5 | 83.60 | 10.00 | 18.90 | 15.10 | 19.53 | 98.66 | 27.4 | 2849.75 | 3415.86 |
| T6 | 86.33 | 11.00 | 22.57 | 17.50 | 20.80 | 99.33 | 28.1 | 2928.47 | 3520.00 |
| T7 | 86.16 | 10.91 | 22.62 | 17.53 | 19.86 | 99.00 | 27.88 | 2904.65 | 3501.43 |
| T8 | 75.70 | 7.90 | 16.00 | 11.80 | 18.93 | 77.00 | 24.8 | 2500.00 | 3005.00 |
| T9 | 79.60 | 10.08 | 16.80 | 11.50 | 19.54 | 93.66 | 27.6 | 2842.75 | 3235.98 |
| T10 | 81.36 | 9.91 | 17.59 | 12.06 | 19.49 | 92.00 | 27.3 | 2797.52 | 3083.60 |
| T11 | 79.10 | 11.13 | 17.05 | 11.51 | 20.82 | 100.00 | 28.2 | 2949.89 | 3251.68 |
| T12 | 79.51 | 11.43 | 17.20 | 11.80 | 20.86 | 102.66 | 28.3 | 3261.00 | 3534.00 |
| T13 | 75.60 | 7.86 | 16.10 | 11.82 | 18.76 | 86.33 | 24.8 | 2485.62 | 2990.66 |
| T14 | 81.73 | 10.19 | 18.03 | 14.80 | 19.10 | 97.00 | 25.5 | 2696.66 | 3240.00 |
| T15 | 82.40 | 10.26 | 19.10 | 15.20 | 19.26 | 99.33 | 25.8 | 2700.11 | 3246.30 |
| T16 | 82.06 | 10.03 | 19.00 | 14.20 | 19.40 | 85.66 | 26.4 | 2664.18 | 3193.93 |
| T17 | 72.93 | 7.53 | 14.80 | 8.50 | 16.70 | 66.66 | 23.2 | 1821.00 | 2193.33 |
| CD 5% | 2.44 | 0.38 | 0.27 | 0.46 | 0.93 | 2.81 | 1.3 | 88.41 | 122.99 |

**Fig 8. Management of bacterial blight disease of rice (Coefficient of infection)**

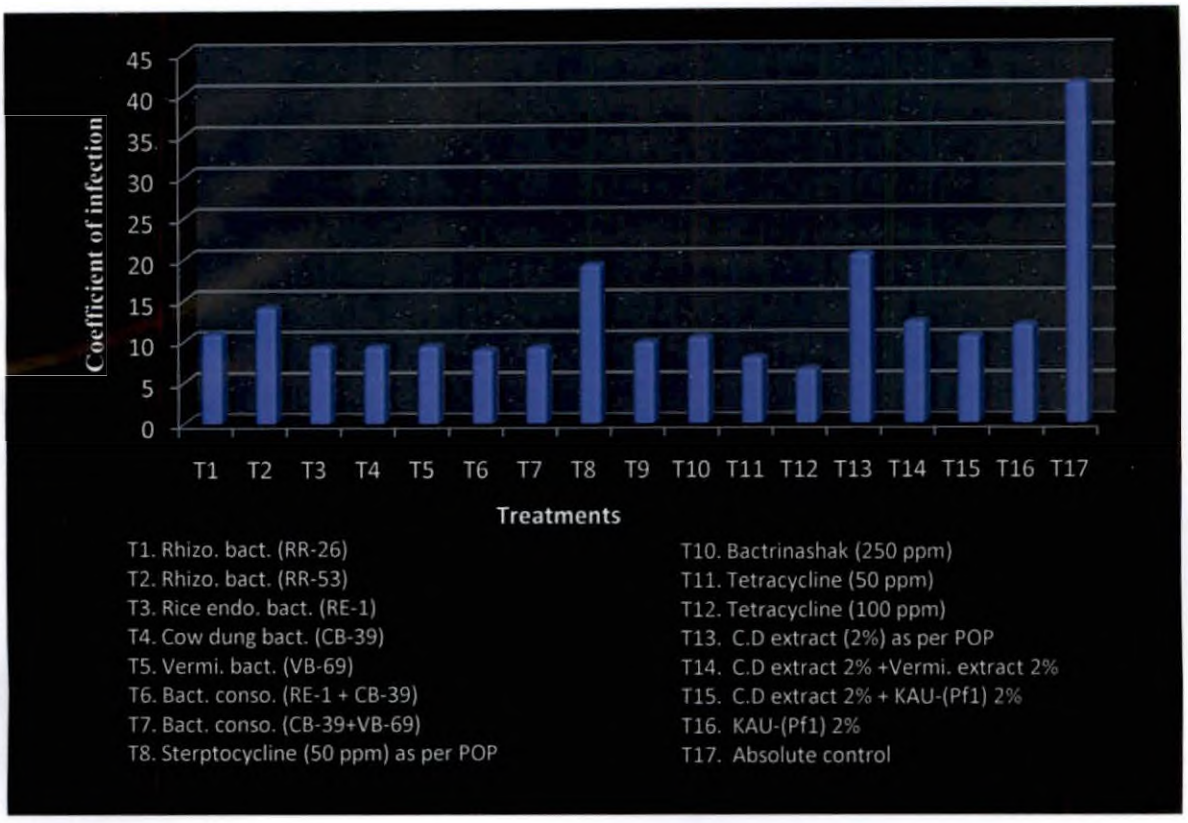
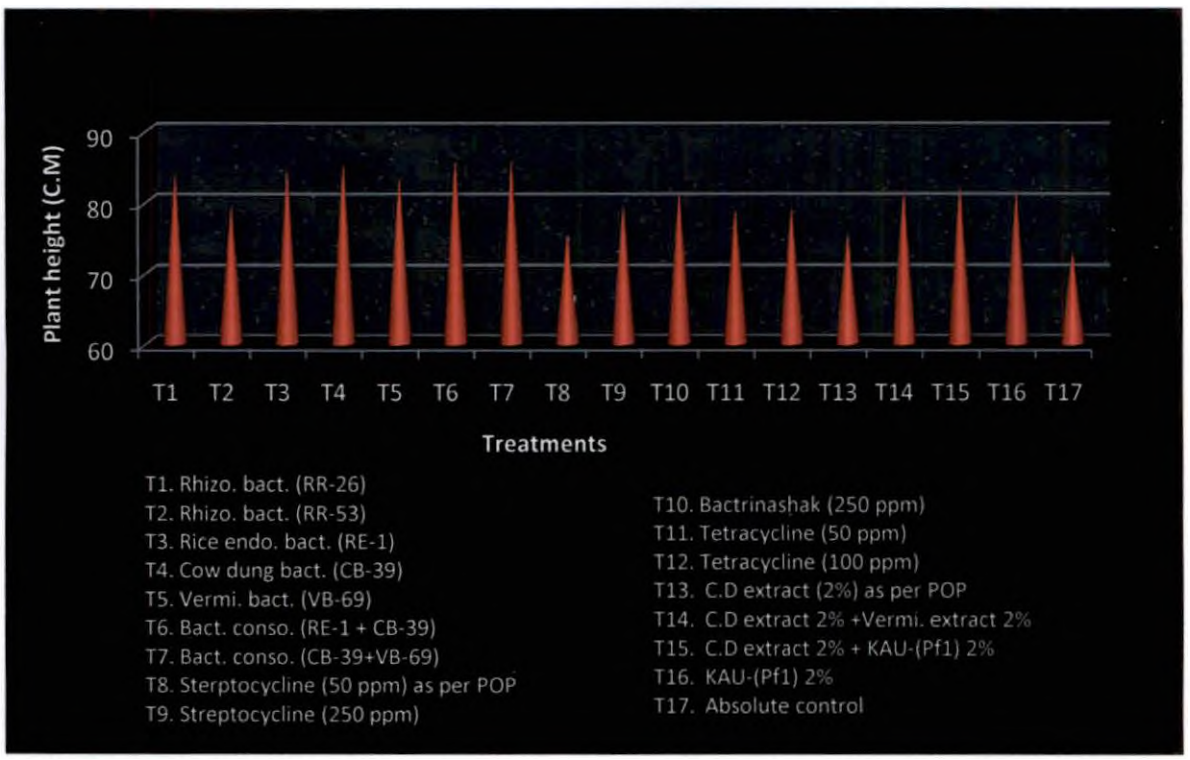



**Fig 9. Effect of different treatments on plant height**



consortium RE-1 + CB-39 (T6) were found significant in increasing the number of tillers with the mean tiller numbers of 11.43, 11.13 and 11 respectively. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4) and endosphere bacteria RE-1 (T3) were found next in increasing the tiller numbers with the mean tiller numbers of 10.91, 10.83 and 10.53 respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + KAU-(Pf1) 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14) and streptocycline 250 ppm (T9) were found on par with the mean tiller numbers of 10.43, 10.26, 10.19 and 10.08 respectively. The treatments *viz.*, KAU-(Pf1) 2% (T16), bacteria from vermicompost VB-69 (T5) and Bactrinashak 250 ppm (T10) were found on par with the mean tiller numbers of 10.03, 10, and 9.91 respectively. The treatment rhizosphere bacteria RR-53(T2) found next with the mean tiller number of 8.80. The treatments *viz.*, streptocycline 50 ppm (T8), cow dung extract 2% (T13) and absolute control (T17) were found least in increasing the tiller number. They recorded the mean tiller number of 7.90, 7.86 and 7.53 respectively.

**4.13.2.1.4. Root length (cm)**

The effect of different treatments on root length was studied at the time of harvest of the crop. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7) and bacterial consortium RE-1 + CB-39(T6) were found superior in increasing the root length with the root length of 22.62cm and 22.57cm respectively (Fig 10). The treatments *viz.*, cow dung extract 2% + KAU-(Pf1) 2% (T15), KAU-(Pf1) 2% (T16), endosphere bacteria RE-1 (T3), bacteria from vermicompost (T5) and rhizosphere bacteria RR-26 (T1) were found next in increasing the root length with the root length of 19.10 cm, 19 cm, 19 cm, 18.90 cm and 18.90 cm respectively. The treatments *viz.*, cow dung extract 2% + vermicompost extract 2% (T14) and rhizosphere bacteria RR-53 (T2) were found on par with the mean root length of 18.03 cm and 17.90 cm respectively. The treatment Bactrinashak 250 ppm (T10) was found next with the mean root length of 17.59 cm. The treatments *viz.*, tetracycline 100 ppm (T12) and tetracycline 50 ppm (T11) were found on par. They showed the mean root length of 17.20 cm and 17.05 cm respectively. The treatment streptocycline 250 ppm was found next with the mean root length of 16.80 cm. The treatments *viz.*, cow dung extract 2% (T13) and streptocycline 50 ppm (T8)

were found on par with the mean root length of 16.10 cm and 16.00 cm respectively. The absolute control (T17) recorded the minimum mean root length of 14.80 cm.

**4.13.2.1.5. Root weight (g)**

The effect of different treatments on root weight was recorded after drying the root samples. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7) and bacterial consortium RE-1 + CB-39 (T6) were found significantly superior in increasing the root weight with the mean root weight of 17.53 g and 17.50 g and respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + KAU-(Pf1) 2% (T15) and bacteria from vermicompost VB-69 (T5) were found next in increasing the root weight with the mean root weight of 15.50 g, 15.20 g and 15.10 and respectively. The treatments *viz.*, endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4) and cow dung extract 2% + vermicompost extract 2% (T14) were found on par. They showed the mean root weight of 15.00g, 14.90 g and 14.80 g respectively. The treatments *viz.*, KAU-(Pf1) 2% (T16) and rhizosphere bacteria RR-53 (T2) were found on par with the mean root weight of 14.20 g and 14.00 g respectively. The treatments *viz.*, Bactrinashak 250 ppm (T10), cow dung extract 2% (T13), streptocycline 50 ppm (T8) and tetracycline 100 ppm (T12) were found next with the mean root weight of 12.06 g, 11.82 g, 11.80 g and 11.80 g respectively. The treatments *viz.*, tetracycline 50 ppm (T11) and streptocycline 250 ppm (T9) were found on par with the mean root weight of 11.51g and 11.50 g respectively. The absolute control (T17) recorded the minimum mean root weight of 8.50 g.

**4.13.3. Yield attributes**

**4.13.3.1. Panicle length (cm)**

The effect of different treatments on mean panicle length showed that the treatments *viz.*, tetracycline 100 ppm (T12), tetracycline 50 ppm (T11), bacterial consortium RE-1 + CB-39(T6), bacteria from cow dung CB-39 (T4) and endosphere bacteria RE-1(T3) were found significantly effective in increasing the panicle length with the mean panicle length of 20.86 cm, 20.82 cm, 20.80 cm, 20.50 cm and 20.16 cm respectively. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7), streptocycline 250 ppm (T9), bacteria from vermicompost VB-69 (T5), rhizosphere bacteria RR-26 (T1), Bactrinashak 250 ppm (T10), KAU-(Pf1) 2%

(T16), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14), and streptocycline 50 ppm (T8) were found next in increasing the panicle length. They showed the mean panicle length of 19.86 cm, 19.54 cm, 19.53 cm, 19.50 cm, 19.49 cm, 19.40 cm, 19.26 cm, 19.10 cm and 18.93 cm respectively. The treatments *viz.*, cow dung extract 2% (T13) and rhizosphere bacteria RR-53 (T2) were found next with the mean panicle length of 18.76 cm and 18.13 cm respectively. The absolute control (T17) showed the mean panicle length of 16.70 cm.

**4.13.3.2. Number of filled grains/panicle**

The effect of different treatments on number of filled grains/panicle revealed that the plots received with treatments *viz.*, tetracycline 100 ppm (T12) and tetracycline 50 ppm (T10) were found significantly superior in increasing the number of filled grains/panicle. They showed the mean filled grain of 102.66 and 100 respectively. The treatments *viz.*, bacterial consortium RE-1 + CB-39 (T6),Cow dung extract 2% + KAU-(Pf1) 2% (T15), tetracycline 50 ppm (T11), bacteria from vermicompost VB-69 (T5) and cow dung extract 2% + vermicompost extract 2% (T14) were found next with the number of filled grains of 99.33, 99.33, 99, 98.66 and 97 respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), streptocycline 250 ppm (T9), bacteria from cow dung CB-39 (T4) and Bactrinashak 250 ppm (T10) were found on par. They showed the mean number of filled grains of 94, 93.66, 92.33 and 92 respectively. The treatments *viz.*, endosphere bacteria RE-1 (T3) and rhizosphere bacteria RR-53 (T2) were found on par with the mean number of filled grains of 90.66 and 88.66 respectively. The treatments *viz.*, cow dung extract 2% (T13) and KAU-(Pf1) 2% (T16) were found on par with the mean number of filled grains of 86.33 and 85.66 respectively. The treatment sterptocycline 50 ppm (T8) recorded the mean number of filled grains of 77. The absolute control recorded the minimum number of filled grains of 66.66.

**4.13.3.3. Thousand seed weight (g)**

The effect of different treatments on the 1000 seed weight showed that the plots which received the treatments *viz.*, tetracycline 100 ppm (T12), tetracycline 100 ppm (T11), bacterial

**Fig 10. Effect of treatments on length and weight of roots**

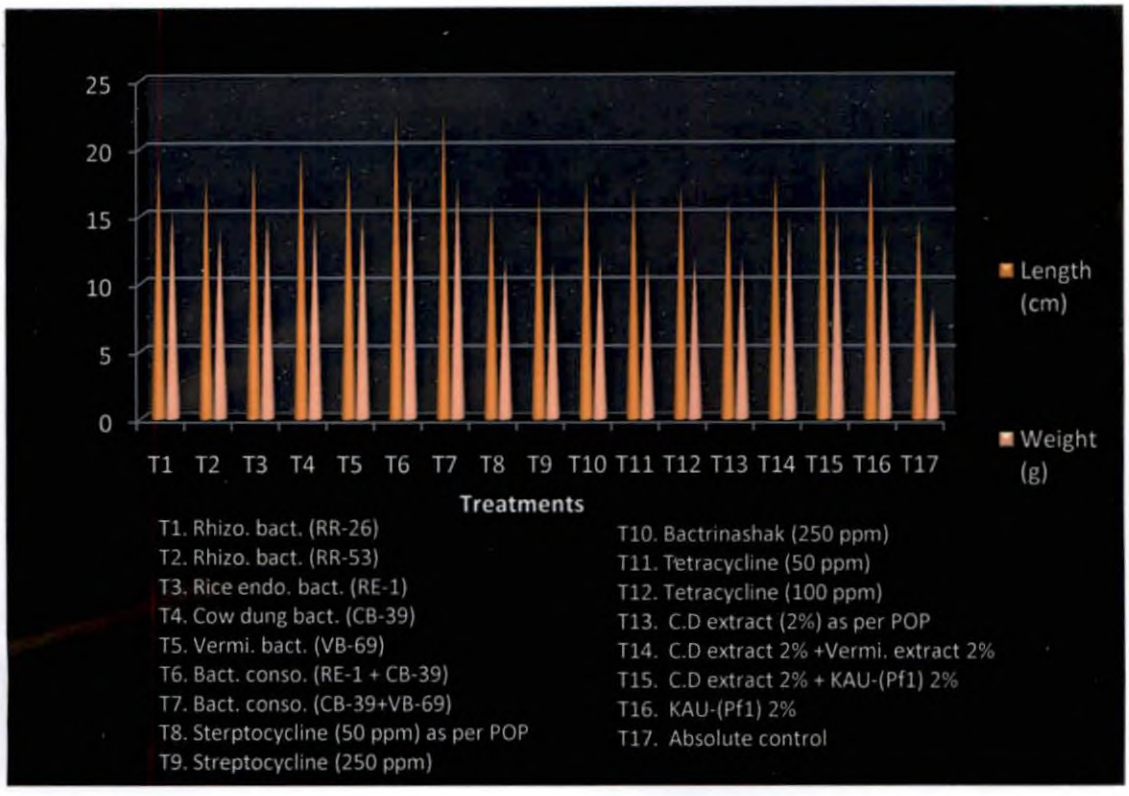
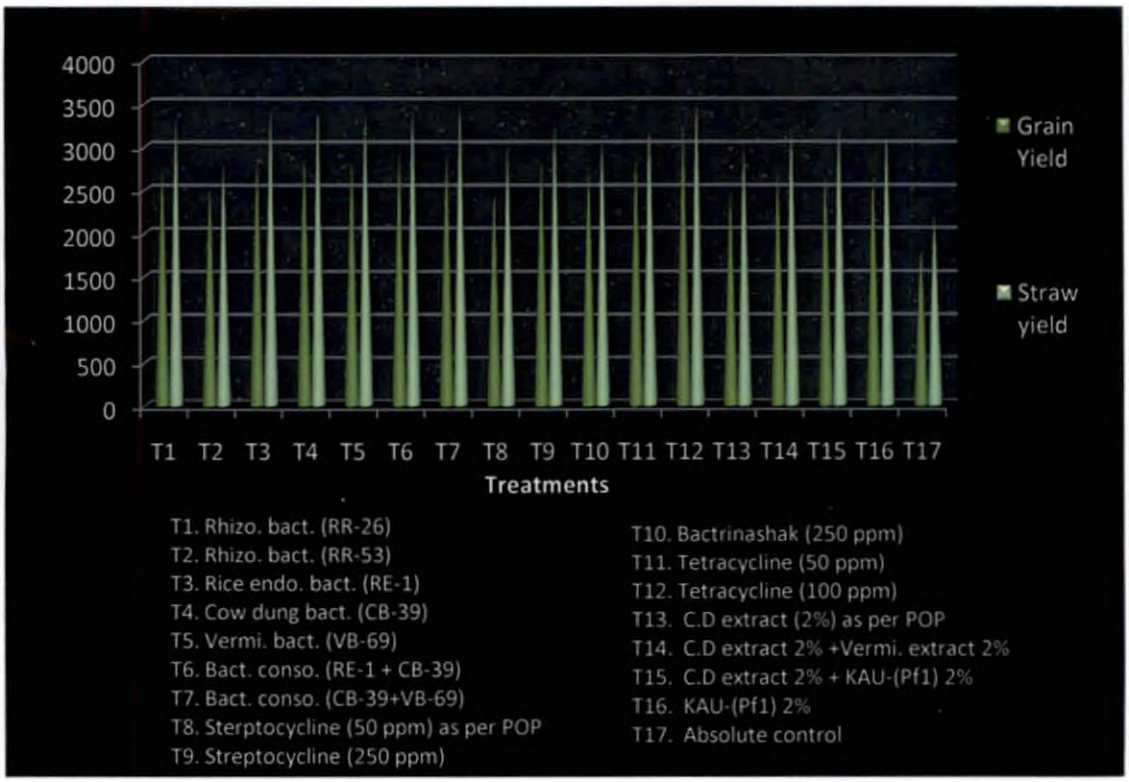



**Fig 11. Effect of treatments on yield of paddy (kg/ha)**



consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1(T3), streptocycline 250 ppm (T9), bacteria from vermicompost VB-69 (T5) and Bactrinashak 250 ppm (T10) were found significantly superior in increasing the seed weight with the mean 1000 seed weight of 28.30g, 28.20g, 28.10g, 27.88g, 27.80g, 27.60g, 27.60g, 27.40g and 27.30g respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), KAU-(Pf1) 2% (T16), cow dung extract 2% + *P. fluorescens* 2% (T15) and cow dung extract 2% + vermicompost extract 2% (T14) were found next in increasing the 1000 seed weight with the mean 1000 seed weight of 26.80g, 26.40g, 25.80g and 25.50 g respectively. The treatment rhizosphere bacteria RR-53 (T2) found next with the mean 1000 seed weight of 25.20 g. The treatments *viz.*, streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par. They showed the mean 1000 seed weight of 24.80g and 24.80g respectively. The absolute control (T17) recorded the minimum mean 1000 seed weight of 23.20g.

**4.13.3.4. Yield (kg/ha)**

The plots which received the treatment tetracycline 100 ppm (T12) recorded significantly higher yield of 3261 kg/ha (Fig 11). The treatments *viz.*, tetracycline 50 ppm (T11), bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7) and bacteria from cow dung CB-39 (T4) were found next in increasing the yield. They recorded the yield of 2949.89, 2928.47, 2904.65 and 2899.89 kg/ha respectively. The treatments *viz.*, endosphere bacteria RE-1(T3), bacteria from vermicompost VB-69 (T5), streptocycline 250 ppm (T9) and Bactrinashak 250 ppm (T10) were found on par with the yield of 2857.04, 2849.75, 2842.75, and 2797.52 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14) and KAU-(Pf1) 2% (T16) were found on par with the yield of 2749.90, 2700.11 , 2696.66 and 2664.18 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-53(T2), streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par with the yield of 2557.03, 2500.00, 2485.62 kg/ha. The absolute control (T17) recorded the yield of 1821 kg/ha.

**4.13.3.5. Straw yield (kg/ha)**

The plots which received the treatments *viz.*, tetracycline 100 ppm (T12), bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), cow dung bacteria CB-39 (T4), endosphere bacteria RE-1(T3) and bacteria from vermicompost VB-69 (T5) were found significantly superior over other treatments in increasing the straw yield. They showed the mean straw yield of 3534, 3520, 3501.43, 3487.76, 3435.28 and 3415.86 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-26(T1), tetracycline 50 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14), streptocycline 250 ppm (T9) and KAU-Pf1 2% (T16) were found on par with the mean straw yield of 3314.28, 3251.68, 3246.30, 3240, 3235.98 and 3193.93 kg/ha. The treatments *viz.*, Bactrinashak 250 ppm (T10), streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par with the mean straw yield of 3083.60, 3005 and 2990.66 kg/ha. The absolute control (T17) recorded the minimum straw yield of 2193.33 kg/ha.

# *Discussion*

# 5. DISCUSSION

Bacterial blight of rice (*Oryza sativa* L.) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is responsible for severe losses to rice crop in tropical, sub tropical and warm temperate regions of the world. Infection of the crop at tillering stage can sometimes lead up to 100 per cent yield loss (Mew and Majid, 1977). To avoid the crop loss due to this devastating disease, an effective management approach is inevitable. The management strategy mainly focused on the application of antibiotics to combat the disease, but this method will not always be effective when an epidemic occurs. Breeding for disease resistance is sound, eco-friendly method, but the variation among the isolates due to change in virulence, often break the resistance level and this method of management is also a challenging task for the breeders. Another avenue in managing the disease is by use of biocontrol agents, which are safer and eco-friendly due to its disease management qualities and growth promoting effect. It was in this background the present investigation was carried out, which consists of survey, collection and isolation of different isolates of *Xoo* from disease prone areas, study on the pathogenic and genetic variability among the different isolates to know the virulence spectrum of pathogen, *in vitro* and *in planta* evaluation of selected bactericides, organics and biocontrol agents against the pathogen, study on the compatibility of efficient antagonists with common plant protection chemicals and effective management of bacterial blight disease of rice. The results emerged from the study are logically discussed hereunder.

## 5.1. Survey on the occurrence of bacterial blight disease in three districts of Kerala

Survey conducted in 14 locations of three districts of Kerala *viz.*, Alappuzha, Palakkad and Thrissur to study the occurrence of bacterial blight disease revealed that, the Per cent Disease Incidence (PDI), Per cent Disease Severity (PDS) and Co-efficient of Infection (CI) ranged from 20.94 – 60.34, 57.05 – 92.42 and 11.97 – 55.77 respectively. The maximum PDI value was recorded in paddy fields of Erattakulam (60.34), Athimani (53.95), Parali (53.87), Polpully (49.77), Nenmara (47.55) and Edathua (46.16). The PDS was also maximum in paddy fields of Erattakulam (92.42), Nenmara (88.63), Athimani (87.65), Parali (87.18), Pattambi (85.72) and Polpully (85.49).

Maximum CI values were recorded from Erattakulam (55.77), Athimani (47.30) and Parali (46.96), Polpully (42.55) and Nenmara (42.13). Based on the PDI, PDS and CI values, it was concluded that out of the 14 rice growing locations surveyed on the occurrence of bacterial blight on two popular varieties *viz.*, Uma (MO-16) and Jyothi (PTB-39), rice fields of five locations *viz.*, Erattakulam, Athimani, Parali, Polpully and Nenmara in Palakkad district recorded susceptible reaction with CI values ranging from 42.13 - 55.77. The rice fields of seven locations *viz.*, Edathua, Kodakara, Mannuthy, Pattambi, Karuvatta, Akamala and Manchira recorded moderately susceptible reaction with CI values ranging from 27.26 - 38.99. Kodallur (12.29) and Moncombu (11.97) fields recorded moderately resistant reaction. This gave a preliminary idea of difference in the virulence of the bacterial pathogen present in these three districts.

The survey was conducted during the vulnerable period of panicle initiation, a stage which is highly prone to bacterial blight development in rice. Heavy incidence of the disease might be due to highly favourable environmental conditions like strong winds, rain splashes, high relative humidity, less temperature and flood due to heavy monsoon during this period. Large scale cultivation of farmer preferred susceptible varieties *viz.*, Jyothi (PTB-39), Uma (MO-16) and Bhadra (MO-4) as monocrop continuously on the same field during *viruppu* (First crop season), *mundakan* (Second crop season) and *punja* (Third crop season) of Kerala might have increased the possibility of perpetuating the pathogen in crop debris left in the field. The practice of heavy fertilization, especially over doses of nitrogenous fertilizers on high nitrogen responsive rice varieties with assured irrigation might have made the disease in an endemic/epiphytotic form in Palakkad district. Raina *et al.* (1981) and Mew *et al.* (1993) opined that the cultivation of high yielding varieties and excessive use of nitrogenous fertilizers are the major reasons for the outbreak of bacterial blight disease. This is further supported by Gnanamanickam *et al.* (1999) with the report that the susceptibility of the cultivar, the stage of the crop and the conduciveness of the environment are the prime factors for the bacterial blight development and subsequent reduction in grain yield. Rajarajeswari and Muralidharan (2006) conducted surveys to assess the damage due to natural epidemics during the period between 1995 to 1998 in Andhra Pradesh and pointed

out that the bacterial blight of rice is more prevalent in both South West (June – September) and North East (October – November) monsoon periods.

## 5.2. Symptomatology of the disease

### 5.2.1. Symptomatology under natural and artificial conditions

During the survey, two phases of bacterial blight *viz.*, kresek and leaf blight were observed. The kresek symptoms were observed on seedlings in the nursery and on main field immediately after transplanting. In nursery, it appeared initially as greyish green leaves followed by rolling along the midrib, withering, wilting and drying of the entire plants. In the transplanted crop, yellowing and stunting of the crop were observed. Leaf blight phase was observed during flowering stage as water soaked lesions on leaf margins near the tip of leaves, followed by yellow lesions on the leaf margins were observed initially. In advanced stage, the characteristic symptom of leaf blight as yellow wavy margins of the leaves leaving a small green portion in the centre was noticed. Similar types of symptoms were also reported by many workers (Tagami and Mizukami, 1962; Ou, 1985; Mew, 1987; Elings *et al.* 1997; Rajarajeswari and Muralidharan, 2006 and Yong, 2010).

Seed inoculation was done on 14 isolates on the susceptible variety Jyothi (PTB - 39) and the seedlings showed the kresek symptom as pale yellow leaves initially followed by rolling along midrib, withering and drying of the entire plants. On artificial inoculation during tillering stage of the crop could produce the leaf blight symptom of yellow wavy lesions on both sides of the leaves. Later the lesions turn papery white and caused drying of the foliage. The present results are in agreement with that of the earlier workers (Muneer *et al.*, 2007 and Jabeen *et al.*, 2012).

## 5.3. Isolation of *Xoo* and pathogenicity test

The causal organism of bacterial leaf blight of rice - *Xanthomonas oryzae* pv. *oryzae* was isolated from the infected leaf samples collected from different places of

Alappuzha, Palakkad and Thrissur districts. The samples from different areas yielded distinct, typical yellow, slimy, convex and circular colonies on potato sucrose agar medium (PSA). Bradbury (1984) observed that the xanthomonads could produce mucoid colonies on glucose containing solid media. Di *et al.* (1991) also had reported that the bacterial blight pathogen on growth factor agar (GFA) were very small, yellow and slimy. Kotasthane (2003) reported that on Wakimoto's medium, the bacterial blight pathogen produced smooth, convex and straw yellow coloured colonies. Jabeen *et al.* (2012) also observed that the bacterial blight pathogen on isolation yielded yellow, mucoid and dome shaped slimy colonies on yeast extract dextrose calcium carbonate agar (YDCA) medium and nutrient agar (NA) medium.

Pathogenicity of all the isolates was proved by inoculating bacterial cell suspension ($10^8$cfu/ml) to the susceptible variety, Jyothi (PTB-39). The characteristic symptoms were observed on rice leaves within five days of inoculation on different isolates, as small, water soaked yellow lesions, with wavy margins on leaf blades which later on developed into papery white in colour. Reisolation of the pathogen from artificially inoculated plants yielded bacterial colonies similar to the original one. Ghasemie *et al.* (2008), while studying the 18 Iranian bacterial blight isolates, also had observed the appearance of pale green to grey green water soaked streaks near the leaf tip and margins of the inoculated plants in the cultivar, Khazar, at 10 to 14 days interval.

### 5.4. Cultural, morphological and biochemical characters of *Xoo* isolates

Cultural and morphological studies were carried out with 14 isolates on PSA medium to find out the variation with respect to colony characters like size, shape, pigmentation, sliminess, colony edge, appearance of colony surface and incubation period. The colony size of the 14 isolates varied from 1-3 mm diameter. Eight isolates *viz.*, Athimani (XAMI-1), Erattakulam (XERM-5), Kodallur (XKOR-3), Manchira (XMRA- 4), Parali (XPAI-5), Akamala (XAKA-4), Mannuthy (XMTY-2) and Karuvatta (XKVA-2) had colonies of 1 to 2 mm diameter, whereas the remaining six isolates *viz.*, Nenmara (XNRA-5), Pattambi (XPTB-2), Polpully (XPLY-3), Kodakara (XKDA-3), Edathua (XEDA-3) and Moncombu (XMOU-2) had colonies of 1 to 3 mm diameter.

Twelve isolates had circular, convex and raised colonies except the two. Manchira (XMRA-4) and Moncombu (XMOU-2) had irregular margin colonies. The isolates differed slightly with respect to colony colour. All the isolates showed dark yellow colonies with good sliminess, except Akamala (XAKA-4) and Edathua (XEDA-3), which were light yellow with moderate sliminess.

Time taken for the appearance of colony *viz.*, incubation period also slightly varied among the 14 isolates. The nine isolates *viz.*, Akamala (XAKA-4), Edathua (XEDA-3), Karuvatta (XKVA-2), Kodakara (XKDA-3), Kodallur (XKOR-3), Manchira (XMRA- 4), Moncombu (XMOU-2), Pattambi (XPTB-2) and Mannuthy (XMTY-2) showed 24 h of incubation for the colony development, where as Athimani (XAMI-3) and Polpully (XPLY-3) isolates took 36 h for the colony appearance. The Erattakulam (XERM-5), Nenmara (XNRA-5) and Parali (XPAI-5) isolates showed 48 h of incubation for the colony appearance. Jabeen *et al.* (2012) reported the appearance of yellow, circular, smooth, convex and viscous bacterial colonies on yeast extract dextrose calcium carbonate agar medium (YDCA) from bacterial blight samples at 48-72h incubation period. In *Xanthomonas oryzae* special (XoS) medium the samples gave light yellow, mucoid, round and smooth bacterial colonies of one mm diameter. Khalid and Sinha (2008) carried out experiments to study the cultural, physiological and biochemical variations among 20 isolates of *Xoo* obtained from rice growing localities in Uttarakhand, Uttar Pradesh, Andhra Pradesh, Chhattisgarh and Orissa, India. They found that all the isolates grew fast on Wakimoto's medium (11.3-18.0 mm after 96 h of incubation). Among them, the isolate *Xoo* 9 (from Rampur, Uttar Pradesh) gave the highest radial growth (18 mm) in all media tested after 96 h of incubation.

Biochemical studies done in the present investigation revealed that all the 14 isolates reacted positively to starch hydrolysis, levan formation and arginine dihydrolase tests and produced pink colour rods on Gram staining and were found Gram negative. All the14 isolates showed positive reaction to KOH test by forming thick thread. This result is confirmative of the Gram negative reaction of these isolates. The isolates differed in citrate utilization, gelatin liquefaction and $H_2S$ production. Parali (XPAI-3) and Pattambi isolates (XPTB-4) could utilize the citrate. Except Kodallur (XKOR-3) and Nenmara

(XNRA-1) isolates, all the other 12 isolates could liquefy gelatin. $H_2S$ was produced by Parali (XPAI-3) isolate alone. Muko and Isaka (1964), using the Japanese isolates, and Goto (1964) using tropical strains had reported gelatin liquefaction, production of ammonia and $H_2S$, alkaline reaction of litmus milk and acid production from some sugars. Hifni *et al.* (1975) while studying 30 isolates from Indonesia and Japan had obtained similar results and more or less confirmed the results of Muko and Isaka (1964) and of Goto (1964). Shekhawat and Srivastava (1968) long back had found two distinct biochemical strain groups in six isolates from India. The first group was similar to normal strains while the second group hydrolysed starch completely; had acid reaction to litmus milk; and was insensitive to penicillin. Reddy and Ou (1976) studied 40 isolates from nine countries of Asia. They also confirmed the results of Muko and Isaka (1964) and of Goto (1964). Muneer *et al.* (2007) while studying the biochemical variability of *Xoo* isolates form Pakistan had found the reaction to be negative for oxidase, lecithinase and Gram's reactions and for the biochemical tests *viz.*, tween 80 and starch hydrolysis and anaerobic nature varied among the isolates. Only 20 per cent of the isolates were similar in terms of their reactions to these tests. Kumar *et al.* (2009) also reported four isolates of *Xoo* from India, showing varying degrees of physiological and biochemical characters. All the above studies are more or less supportive of the results obtained in the present study.

Almost all the isolates produced acid from one or the other carbon sources used in this study. All the isolates produced acid from lactose and sucrose. Only Polpully isolate (XPLY-5) produced acid from xylose and maltose. Kodakara isolate (XKDA-1) alone could produce acid from fructose. Manchira (XMRA-2) and Polpully (XPLY-5) isolates produced acid from galactose, raffinose, trehalose and melibiose. The isolates from Kodallur (XKOR-3), Manchira (XMRA-2) and Polpully (XPLY-5) could produce acid from L-arabinose. Polpully (XPLY-5) alone could produce acid from mannose. *Xoo* is a weak producer of acids from carbohydrates is in conformity with Bradbury (1984). Khalid and Sinha (2008) also reported that the *Xoo* isolates varied in their ulilization of carbon source and xylose was the most preferred carbon source followed by sucrose, fructose, glucose, arabinose and mannose.

Cultural, morphological and biochemical studies of 14 isolates collected from three districts of Kerala *viz.* Alappuzha, Palakkad and Thrissur revealed that, variations existed among the isolates of *Xoo*. Based on these characters, the pathogen causing bacterial blight disease was tentatively identified as *X. oryzae* pv. *oryzae*.

### 5.5. Studies on the pathogenic variability of *Xoo* isolates

This part of study is a highly perspective aspect of practical importance. Knowledge on the pathogenic variability of *Xoo* isolates is necessary to exploit genetic pools for developing rice with the durable resistance to bacterial blight. The effective use of genetic resources for bacterial blight resistance requires efficient characterization of pathogen populations and careful selection of R genes with suitable deployment strategies. With this in view, pathogenic variability of 14 isolates was studied on 20 cultivated rice varieties, six near isogenic lines and on three rice differentials in net house.

Pathotype study of 14 isolates on 20 varieties, established that the Palakkad isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed susceptible reaction on 19 cultivated rice varieties and resistant reaction on rice variety Makaram (KTR-2) were grouped as highly virulent. The isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) isolates showed moderately susceptible reaction to 19 rice varieties and resistant reaction on rice variety Makaram and were grouped as moderately virulent. The isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed moderately resistant reaction on nineteen rice varieties and resistant reaction on rice variety Makaram and hence were grouped as weakly virulent. The photosensitive long duration variety Makaram (a bulk progeny selection from local Cherady) might have got the resistant gene/genes that confer the resistant reaction to all the 14 isolates collected from the three major rice growing districts of Kerala. Further molecular study is essential to elucidate the genes responsible for the resistance in Makaram. The present study passes a practical message to the plant breeders that the variety Makaram may be used for breeding

programmes to develop a stable broad spectrum of resistance to bacterial blight in rice cultivars. The highly virulent isolates identified in this study can be used for screening large germplasm to find out the resistant donors for the breeding programmes. Similar type of resistance in the cultivar, Laxmi which offered high level of resistance was reported by Adhikari and Mew (1994). Artificial inoculation studies done by Tyagi *et al.* (2010) established that eight Indian land races from Bihar and Jharkhand *viz.*, Bhathani, Hardi Muril, Sitwadhan, Jhulat, Lamb Asari, Karijiri, Swarna Gora and Sita Gora were found highly resistant to bacterial leaf blight pathogen. Thus, the present investigation put forth the practical opportunities and challenges to plant pathologists, rice breeders and biotechnologists to find out the resistant gene present in the variety Makaram.

Pathotype study of 14 isolates on six near isogenic lines and three rice differentials also indicated the similar spectrum of virulence. The isolates from Palakkad district *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed moderately susceptible reaction on IRBB-4 (*Xa4*), IRBB-5 (*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) and IRBB-57 (*Xa4/xa5/Xa21*). But they showed resistant reaction on IRBB-60 (*Xa4/xa5/xa13/Xa21*) and susceptible reaction on the three rice differentials *viz.*, Ajaya, IR-8 and IR-24. These isolates were classified as 'highly virulent isolates'. The seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) showed moderately resistant reaction on IRBB-4 (*Xa4*), IRBB-5 (*xa5*), IRBB-13 (*xa13*), IRBB-21 (*Xa21*) and IRBB-57 (*Xa4/xa5/Xa21*) and resistant reaction on IRBB-60 (*Xa4/xa5/xa13/Xa21*) and moderately resistant to moderately susceptible reaction on Ajaya and susceptible reaction on IR-8 and IR-24. In the present study, these seven isolates mentioned above are classified as 'moderately virulent isolates'. The isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed resistant reaction on all the six near isogenic lines and on rice differentials and thus form a different group of 'weakly virulent isolates'. According to Kumar *et al.* (2009) the *Xo*-A and *Xo*-B isolates from India expressed more severe symptoms than *Xo*-C and *Xo*-D isolates. The present results of variation in the virulence level of the pathogen population in Kerala are in agreement with the previous workers

(Lore *et al.*, 2011 and Chen *et al.*, 2012). According to them, the bacterial blight isolates from India (Punjab) and China were highly variable and they classified them in to virulent and least virulent pathotypes.

The present study has offered useful practical information on the variability of the pathogen population ('highly virulent', 'moderately virulent' and 'weakly virulent') in Kerala. The near isogenic line IRBB-60 which contains two major dominant resistant genes (*Xa4* and *Xa21*) and two recessive genes (*xa5* and *xa13*) offer both qualitative and quantitative components of resistance and these genes are involved in different defense pathways in rice as opined by Li *et al.* (2001) and can give broad spectrum of resistance to different pathogen populations of Kerala. The present study also revealed that, the pathogen population in Kerala can break the durability of *Xa4*, *xa5*, *xa13* and *Xa21*, the major genes offering resistance, when they are used individually in the breeding programme. The line IRBB-60 offered high level of resistance against bacterial blight isolates from Vietnam (Loan *et al.*, 2006).The wider level of resistance against *Xoo* strains across different parts of India was also reported by Bharathkumar *et al.*, (2008). The isolates from Kerala, on inoculation on IRBB-60 offered highly resistant to resistant reaction (Perumalsamy *et al.*, 2010; Sujatha *et al.*, 2011). Rice line NH56 carrying R genes (Xa4+xa5+xa13+Xa21) were found resistant against isolates of the pathogen obtained from Kerala was also reported by Priyadarisini and Gnanamanickam (1999). The present finding on gene combination gives a potential message to the breeders for the marker assisted selection programme (MAS) in Kerala for combating bacterial blight disease. This part of the present study on characterization and pathogenic variability of bacterial blight of rice is the first of its kind of authentic nature in Kerala State.

The kresek symptom of bacterial blight is a systemic phase of the disease and it is otherwise called as wilting syndrome, in which the young plants show the symptoms of pale yellowing, stunting followed by wilting due to vascular infection caused by the bacterium. The study on kresek symptom on 20 varieties done in the present study also showed the similar trend of variability among the isolates of the pathogen. The five isolates from Palakkad *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara

(XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) showed susceptible reaction on all 20 varieties. The seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (XPTB-4) isolates showed moderately susceptible reaction on all the 20 varieties. The two isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) showed resistant reaction on all 20 rice varieties. Reddy and Mohanty (1981) reported that dipping of roots of the rice seedlings for a period of five minutes could cause kresek symptom in rice cultivars Co-33, IR-8 and TN-1. They also observed that the Indian isolates from different areas varied in their ability to induce kresek symptom. The young plants were susceptible than the older ones. The fact that virulent isolates can cause kresek symptom in young seedlings, is in agreement of the results already reported by Watanabe (1975) in Sri Lanka; Tabei (1977) in Japan and Hsieh (1978) in Taiwan. The study on kresek symptom is highly essential to distinguish the nitrogen deficiency symptoms and the symptom due to bacterial blight pathogen in the young seedlings which necessitates the application of management practices at the right time to avoid the huge loss occurring after transplanting. The study on kresek phase of bacterial leaf blight of rice with different isolates is also the first work done in Kerala.

Highly virulent isolates require only very less incubation period (2 to 3 days) where as moderately virulent and weakly virulent isolates require three to 15 days for symptom expression. This is due to the increased amount of inoculum build up every year due to the presence of the pathogen on the infected seeds, rice stubbles and harboring of pathogen on weedy graminaceous hosts. The mono cropping system of paddy cultivation also contributes to the build up of pathogen. The weather factors such as high relative humidity and high rainfall during the months of August to December also favour the pathogen in an endemic form, year after year in the same location. There were earlier reports that rice crops raised during monsoon season suffered yield losses as high as 50 per cent (Rangaswamy and Rajagopalan, 1973).

**5.6. Studies on the genetic variability of *Xoo* isolates from three districts of Kerala**

With regard to genetic studies of the 14 isolates of *Xoo* from three districts of Kerala, the BOX and ERIC-PCR fingerprinting analysis has shown the existence of genetic variability among the isolates. The dendrogram for pooled data of 14 isolates revealed the presence of high variations among the 14 isolates of *Xoo*. When 80 per cent similarity was used as limit of discrimination, the isolates *viz.*, Manchira (XMRA-2), Polpully (XPLY-5) and Moncombu (XMOU-1) clustered tightly showing more than 90 per cent similarity. Similarly, the isolates *viz.*, Pattambi (XPTB-4) and Edathua (XEDA-3) showed more similarity (94%) and tightly clustered. Remaining isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Kodallur (XKOR-3), Nenmara (XNRA-1), Parali (XPAI-3), Akamala (XAKA-2), Kodakara (XKDA-1), Mannuthy (XMTY-2) and Karuvatta (XKVA-1) showed very high variability (less than 80% similarity) within themselves and with other isolates each other. This shows the high level of genetic diversity existed among the pathogen population in the three districts of Kerala. Nayak *et al.*, (2008) studied 52 strains of *Xoo* collected from 12 rice growing states of India, were grouped into 13 clusters based on genetic distance. Yong *et al.*, (2011) also reported that both ERIC and BOX primers depicted the existence of extensive genetic variability of 103 *Xoo* isolates obtained from China. Shahrestani *et al.*, (2012) also studied the genetic variability of 60 *Xoo* isolates from Iran using the RAPD markers and found the presence of greater genetic variation in the pathogen population and grouped them into three clusters at a similarity index of 0.6. The molecular level variability confirms that the pathogen population in Kerala is highly diverse and this gives the supportive for the finding of the pathogenic variability study which depicted the difference in the virulence spectrum of the pathogen population in Kerala. The molecular level studies on bacterial blight pathogen in Kerala also the first work done authentically.

**5.7. Management of bacterial blight of rice**

In present day agriculture, the scientists, farmers and general public all over the globe are in the concept of managing the disease through environment-friendly method, which consists use of less chemicals and wider use of organics and biocontrol agents to

avoid the pollution and the chemical residues in the ecosystem, thus promoting the organic farming approaches. Joining this trend, the present study had a strong mandate of exploring different bacterial blight management options comprising antibiotics, different agrochemicals and organics.

### 5.7.1. *In vitro* sensitivity of different bactericides against highly virulent isolates of *Xoo*

Tetracycline 250 ppm was found to be the best of all the bactericides which had shown maximum inhibition on the growth of the pathogen under *in vitro* followed by tetracycline 100 ppm and tetracycline 50 ppm. This was closely followed by streptocycline 250 ppm, streptocycline 200 ppm and Bactrinashak (2-Bromo-2 Nitro Propane-, 3-Diol) 250 ppm. The antibiotics were found highly effective against Athimani and Nenmara isolates followed by Parali and Polpully isolates of the pathogen. The inhibition of the bacterial blight pathogen by streptocycline was also reported by previous workers (Desai *et al.*, 1967., Mahto *et al.*, 1988 and Thimmegowda *et al.*, 2012).The present finding of tetracyline in inhibiting the *Xoo* was in agreement with the previous work done by Balaraman and Rajagopalan (1978). They found that among the antibiotics tried, tetracycline gave maximum inhibition. Bactrinashak in managing bacterial leaf blight of rice was in conformity with the finding of Abdul and Sinha (2007). Antibiotics are known to control plant diseases by acting on the parasites or on the hosts directly (Dutta, 1978). In the present study, the inhibition of the antibiotics was due to direct action of the agents on the pathogen.

### 5.7.2. *In vitro* sensitivity of different organics and agrochemicals against *Xoo*

In another *in vitro* study, two organics alone (cow dung 2% and vermicompost 2%), combination of organics (cow dung extract 2%+ vermicompost extract 2%), commercial biocontrol agent (Pf1) 2% alone, combination of organics and biocontrol agent (cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2%), two chemicals alone (copper oxychloride 500 ppm, copper hydroxide

0.2% ) and a chemical and an antibiotic combination (copper oxychloride 500 ppm + streptocycline 150 ppm) were tested against the highly virulent isolate of Polpully (XPLY-5).

The cow dung extract 2% + *P. fluorescens* 2% and cow dung extract 2% + vermicompost extract 2% were found to be the best in inhibiting the growth of the pathogen. This was closely followed by vermicompost extract 2% + *P. fluorescens* 2%. Vermicompost extract 2 % alone and copper hydroxide 0.2% alone were the next best treatments. The inhibition of *Xoo* by *P. fluorescens* under *in vitro* condition is in conformity with the finding of Manav and Thind (2002). According to them two rhizobacteria *viz.*, *B.subtilis* and *P. fluorescens* could inhibit the bacterial blight pathogen *Xoo* under *in vitro*. Gangwar and Sinha (2010) also reported that the 10 fluorescent pseudomonad isolates showed the inhibition zones of more than 10 mm against the *Xoo* under *in vitro*. According to Mondal *et al.* (2010) *P. fluorescens* strain MBPF-01 alone or in combination with nanocopper could inhibit *Xoo* under *in vitro*. The inhibition of *Xoo* by cow dung extract is in conformity of Murugan *et al.* (2012).They found that the cow urine extract, could inhibit the bacterial blight pathogen with the inhibition zone of 10 to 13 mm under *in vitro* condition. The higher inhibition caused by cow dung extract and *P. fluorescens* might be due to the synergistic action of the antagonistic microbes present in the cow dung as well as the inhibitory effect of *P. fluorescens*. Similarly, in the case of cow dung extract and vermicompost extract, the effect may be due to the combined effect of the microbes present in both the organics. The role of vermicompost extract in inhibiting the pathogen, emerged from the present study, is the first report on managing the bacterial blight pathogen in rice.

### 5.7.3. Enumeration of microbial population and screening of bacterial isolates from rhizosphere, endosphere, cow dung and vermicompost against *Xoo*

In the present study, attempts were made to select antagonistic bacteria prevalent in the rice rhizosphere and endosphere of different rice growing areas of Alappuzha, Palakkad and Thrissur districts of Kerala. For this, a total of 56 soil samples were

collected from the rhizosphere of healthy plants from diseased rice fields and endosphere bacteria from 56 healthy plants from diseased area. Bacterial colonies from rhizosphere, endosphere, cow dung and vermicompost were isolated. Results of the study revealed rich abundance of bacteria in rhizosphere, endosphere and in two organic sources. Jeyarajan *et al.* (1994) observed more abundance of bacteria in suppressive soils than that of conducive ones. Jubina and Girija (1998), while studying the microflora of rhizosphere soil of black pepper, also observed the abundance of soil bacteria. The work of Thankamony (2005) on quantitative estimation of microbial population in vermicompost revealed that bacteria ($104.3 \times 10^5$), fungi ($44.33 \times 10^4$), actinomycetes ($48.48 \times 10^5$), nitrogen fixing bacteria ($19.02 \times 10^3$) and phosphate solubilizing bacteria ($5.51 \times 10^9$) were present in each gram of the compost.

Preliminary screening of 110 bacterial isolates obtained from rhizosphere, endosphere, cow dung and vermicompost was carried out for their antagonistic activity against the virulent Polpully isolate (XPLY-5). Among them, 18 were found to possess antagonistic property against the pathogen. The cow dung bacterium (CB-39) from Pattambi had shown the maximum inhibition zone of 3cm followed by rhizosphere bacteria from Nenmara (RR-26) with the inhibition zone of 2.50cm. The bacterial isolates from vermicompost (VB-67 and VB-69) stood next with the inhibition zone of 2.30 cm each. A rhizosphere bacterium from Pattambi (RR-53) had shown the inhibition zone of 2.20 cm which was followed by the endosphere bacteria from Kodallur *viz.*, RE-1 with the inhibition zone of 2.10. The six bacterial isolates which showed high inhibition zone in the preliminary screening *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67 and VB-69 along with reference culture Pf-1 were tested individually against *Xoo* under *in vitro*. Among the different bacterial isolates, bacteria obtained from cow dung (CB-39) showed the maximum inhibition zone of 6.5 cm, followed by rhizosphere bacteria RR-26 with the inhibition zone of 6.2 cm. Vermicompost bacteria *viz.*, VB-67 and VB-69 ranked next with the inhibition zone of 6 cm each. The reference culture Pf-1 showed the inhibition zone of 5.5 cm. RR-53 and RE-1 showed the inhibition zone of 5 cm and 4.2 cm respectively.

The antagonism index (AI) was the highest for CB-39 (5281.25) followed by RR-26 (4805), VB-67 (4500), VB-69 (4500), Pf1 (3781.25), RR-53 (3125) and RE-1 (2205). Similar results were reported for other pathosystems by various workers (Piexoto *et al.*, 1995; JianHua *et al.*, 1996; Silveira *et al.*, 1996; Yungchun *et al.*, 1997; Manimala, 2003) where they observed variation in the antagonistic reaction of different *Pseudomonas* spp. against *R. solanacearum.* According to Raupach and Kloepper (1998), the lytic activity by the rhizobacterial antagonists against the pathogen is mainly due to their production of lytic enzymes or by inhibitory metabolites. Velusamy and Gnanamanickam (2003) also reported that 27 rhizobacterial strains could produce 2,4-DAPG that could inhibit the *Xoo*. According to Gangwar and Sinha (2010) minimum mean radial growth of *Xoo* (4.3 mm) was recorded with non-volatile compounds produced by fluorescent pseudomonas strain Pf 83. Chung and Hotink (1990) identified *Bacillus* sp., *Enterobactor* sp., *Flavobacterium balustinum*, *Pseudomonas* sp. and *Streptomyces* sp. as biocontrol agents in compost. The present study on the inhibition of *Xoo* under *in vitro* by the bacteria obtained from vermicompost (*Pseudomonas* sp) is a new report.

### 5.8. Characterization of promising bacterial isolates

Cultural characteristics of promising bacterial isolates were studied on nutrient agar medium. The bacterial isolates, RE-1, RR-26, CB-39 and RR-53 showed 2 to 3 mm, white, circular shaped colonies, whereas VB-67 and VB-69 produced 2 to 3 mm, white to light brown, circular colonies. All the bacterial isolates were found to be short rods based on Gram staining.

Biochemical characters of promising bacterial isolates were studied using HIMEDIA KB002 HiAssorted™ Biochemical Test Kit and other 11 routine biochemical tests. All the six bacterial isolates showed positive reaction for citrate utilization which was evident by the change of initial green colour of medium to blue colour. The isolate VB-67 showed positive reaction to lysine decarboxylase. The isolates RE-1, VB-67 and VB-69 showed positive reaction to ornithine decarboxylase. The isolates RR-53 and VB-69 showed positive reaction to urease test. Only VB-69 gave positive reaction to nitrate

reduction test. The isolates RE-1 and VB-69 showed positive reaction to glucose utilization. The isolates, RE-1, VB-67 and VB-69 showed positive response to adonitol utilization. The isolates, RE-1, RR-53, VB-67 and VB-69 showed positive response to lactose utilization. All the six isolates showed positive response to arabinose utilization. Except VB-67, all the five isolates were found positive to sorbitol utilization test. All the six isolates produced pink colour rods on gram staining and were found gram negative. All the six isolates could hydrolyse starch by producing colourless zone in contrast to the blue background of the medium around the bacterial growth on addition of iodine solution. The isolates RE-1, CB-39, VB-67 and VB-69 could liquefy gelatin.

All the isolates produced white domed and mucoid colonies indicating positive reaction for levan production. All the six isolates showed pink colourisation to the medium. Thus shows their ability to hydrolyse arginine. All the isolates showed positive reaction to KOH test by forming thick thread. This observation further confirmed the Gram negative reaction of these isolates. All the bacterial isolates showed positive reaction to trytophan utilization. All the bacterial isolates produced effervescence when hydrogen peroxide was added to the cultures. This indicates the production of catalase. Based on cultural, morphological and biochemical characters these six Gram negative isolates tentatively were identified as *Pseudomonas* spp. and were selected for further studies. Finally it was concluded that all of them were Gram negative short rods and were positive to catalase and arginine dihydrolase activity which clearly confirmed that the isolates belonged to the genus *Pseudomonas* as suggested in the Bergy's Manual of Systematic Bacteriology, Vol I (Staley *et al.*, 1989).

### 5.9. *In planta* evaluation of antagonists, organics and agrochemicals against *Xoo*

The ultimate aim of any studies on plant disease is to have a management system thereby the loss occurring to the farmers can be minimized. Nowadays, due to the awareness on pollution and non target effects of plant protection chemicals, a lot of emphasis is being given to non chemical means of plant disease management, including biological agents and organics. With this practical outlook, the selected six promising

bacterial isolates along with the reference culture, Pf1 were tested in pot culture to assess their efficacy in promoting the growth of rice. The other promising agrochemicals/organics found superior under *in vitro* were also included to test their efficacy in managing the rice bacterial blight pathogen as part of an integrated package. The promising bacterial isolates were inoculated by seed bacterization, seedling dip and also applied as foliar sprays at 30 and 60 DAT. The bactericides were administered as seed treatment and foliar spray. Altogether 21 treatments were included for the study.

**5.9.1. Effect of different antagonists, organics and agrochemicals in management of bacterial blight disease**

Tetracycline 250 ppm recorded the least per cent disease incidence (33.33) and was found to be superior followed by tetracycline 100 ppm (39.99) in checking bacterial blight. The pots received rhizosphere bacteria RR-26 (T1), endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), tetracycline 50 ppm(T7), streptocycline 250 ppm (T11), Bactrinashak 250 ppm (T12), cow dung extract 2% + vermicompost extract 2% (T15), cow dung extract 2% + *P. fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17) and KAU-Pf1 (T20) ranked in that order with PDI value of 46.66. The treatments *viz.*, rhizosphere bacteria RR-53 (T2), streptocycline 200 ppm (T10), cow dung extract 2 % (T13) and vermicompost extract 2% (T14) recorded the PDI value of 53.32 and were on par, where as the treatment bacteria from vermicompost (T5) treated plants recorded the PDI value of 59.99.

The per cent disease severity (PDS) varied from 18.51 to 46.89. Tetracycline 250 ppm (T9) ranked first with PDS value of 18.51 followed by tetracycline 100 ppm (T8) in managing the disease. Streptocycline 250 ppm (T10), tetracycline 50 ppm (T7), Bacterinashak 250 ppm (T12), streptocycline 200 ppm (T10), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6) and endosphere bacteria RE-1 (T3) were also found to be effective and were on par with PDS values of 19.35, 19.43, 19.45, 19.48, 19.58, 19.73 and 19.78 respectively.

The CI values varied from 7.68 to 46.89. Tetracycline 250 ppm(T9) was found to be the best in checking the disease followed by the other treatments *viz.*, tetracycline 100 ppm (T8), streptocycline 250 ppm (T11), Bactrinashak 250 ppm (T12),tetracycline 50 ppm (T7), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), endosphere bacteria RE-1 (T3), rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P. fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17) and cow dung extract 2% + vermicompost extract 2% (T15). The treatments *viz.*, rhizosphere bacteria RR-53 (T2) and the plants which received copper hydroxide 0.2% (T19) ranked next in checking the disease.

The effect of antibiotics in managing the disease may be due to direct action on the pathogen or undergoing transformation within the plants. (Dutta,1978). Bactrinashak (2-Bromo-2 Nitro Propane-1,3-Diol) is an immune modulator, when used as prophylactic treatment reduces the susceptibility of plant to bacterial diseases, by altering the immune system of plants (changing the contents of phenols, proteins, nitrogen and certain enzymes) and make the plants resistant to bacterial attack. Spraying fresh cow dung extract (20 g/l water) has been a proven popular recommendation in Kerala (KAU, 2011).The suppression of disease may be due to the beneficial microflora present in the cowdung. Similarly the increased effect of both cow dung extract and vermicompost extract may be due to the antibacterial microbes present in them (Thankamony,2005).The fluorescent pseudomonads in inhibiting the disease may be due to induction of systemic resistance or due to the production of defense enzymes, as supported by Vidhyasekaran *et al.* (2001). Efficacy of Bactrinashak in managing bacterial blight disease is the first report in Kerala.

### 5.9.2. Effect of antagonists, organics and agrochemicals on biometric characters

The effect of different treatments on biometric characters *viz.*, plant height, number of tillers were studied at 60 DAT where as the yield attributes and the root length and root weight were studied at the time of harvest.

The plants which received treatments *viz.*, rhizosphere bacteria RR-26 (T1), endosphere bacteria RE-1 (T3), vermicompost extract 2% + *P. fluorescens* 2% (T17), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T6), KAU-Pf1(T20), rhizosphere bacteria RR-53 (T2) and cow dung extract 2% + vermicompost extract 2% (T15) were found to be superior in increasing the plant height than the control. They recorded the mean plant height of 88.82 cm, 88.16 cm, 87.74 cm, 87.24 cm, 83.70 cm, 83.70 cm, 83.21 cm, 81.38 cm and 80.48 cm respectively and found to be superior among the treatments where as the control recorded the mean plant height of 57.65 cm only.

The effect of different treatments on mean number of productive tillers showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), bacteria from vermicompost VB-69 (T6), tetracycline 100 ppm (T8), tetracycline 50 ppm (T7), bacteria from cow dung CB-39 (T4), rhizosphere bacteria RR-53 (T2), endosphere bacteria RE-1 (T3), vermicompost extract 2% + *P. fluorescens* 2% (T17), streptocycline 250 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T16), Bactrinashak 250 ppm (T12), rhizosphere bacteria RR-26 (T1) and KAU-Pf1(T20) were found to be significantly superior among the treatments. They recorded the mean number of 16.08, 16.07, 15.99, 15.92, 15.67, 15.67, 15.62, 15.58, 15.52, 15.47, 15.33, 15.30 and 15.10 productive tillers respectively where as the control recorded with the mean productive tiller number of 5.66.

The effect of different treatments on mean root length showed that the plants which received the treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from cow dung CB-39 (T4), vermicompost extract 2% + *P. fluorescens* 2% (T17), KAU-Pf1(T20), cow dung extract 2% + vermicompost extract 2% (T15), rhizosphere bacteria RR-53 (T2) and tetracycline 100 ppm (T8) were found significantly on par in increasing the root length. They showed the mean root length of 18.02 cm, 17.98 cm, 17.74 cm, 17.70 cm, 17.10 cm, 17.08 cm, 17.04 cm and 16.30 cm respectively where as the control recorded with the mean root length of 12.74 cm.

The effect of different treatments on mean root weight showed that the plants which received the treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P. fluorescens* 2% (T16), vermicompost extract 2% + *P. fluorescens* 2% (T17), bacteria from vermicompost VB-69 (T6), cow dung extract 2%+ vermicompost extract 2% (T15), rhizosphere bacteria RR-53 (T2), bacteria from cow dung CB-39(T4), endosphere bacteria RE-1 (T3) and KAU-Pf1 (T20) were found significantly on par in increasing the root weight. They showed the mean root weight of 13.50g, 13.48g, 13.38g, 13.16g, 13.07g, 12.98g, 12.90g, 12.01g and 12.00 g respectively where as the control recorded the mean root weight of 5.72 g.

The effect of different treatments on mean panicle length showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), bacteria from vermicompost VB-69 (T6), rhizosphere bacteria RR-26 (T1), tetracycline 100 ppm (T8), Bacterinashak 250 ppm (T12), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from cow dung (T4), streptocycline 250 ppm (T11), vermicompost extract 2% + *P. fluorescens* 2% (T17), endosphere bacteria RE-1 (T3), tetracycline 50 ppm (T7), cow dung extract 2% + vermicompost extract 2% (T15), KAU-Pf1(T20) and rhizosphere bacteria RR-53 (T2) were found significantly on par in increasing the panicle length. They showed the mean panicle length of 24.86, 24.75, 24.74, 24.55, 24.46, 24.35, 24.30, 24.28, 24.21, 24.20, 24.10, 24.08, 24.09 and 24.00 respectively where as the control showed the minimum mean panicle length of 17.34.

The effect of different treatments on mean filled grains/panicle showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), tetracycline 100 pm (T8), cow dung extract 2% + *P. fluorescens* 2% (T16), cow dung extract 2% + vermicompost extract 2% (T15), vermicompost extract 2% + *P. fluorescens* 2% (T17), tetracycline 50 ppm (T7), bacteria from vermicompost VB-69 (T6), streptocycline 250 ppm (T11) and rhizosphere bacteria RR-53 (T2) were found significantly increasing the number of filled grains per panicle. They showed the mean number of filled grains per panicle of 95.44, 90.32, 86.70, 86.04, 85.92, 85.32, 82.84, 82.80 and 82.06 respectively where as the control showed the mean filled grain number of 59.34.

The effect of different treatments on mean 1000 seed weight showed that the plants which received the treatments *viz.*, tetracycline 250 ppm (T9), rhizosphere bacteria RR-26 (T1), vermicompost extract 2% + *P.fluorescens* 2% (T17), tetracycline 100 ppm (T8), endosphere bacteria RE-1(T3), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from vermicompost VB-69 (T6), Bactrinashak 250 ppm (T12), rhizosphere bacteria RR-53 (T2), cow dung extract 2% + vermicompost extract 2% (T15), streptocycline 250 ppm (T11), KAU-Pf1(T20), tetracycline 50 ppm (T7), streptocycline 200 ppm (T10) and bacteria from cow dung CB-39 (T4) were found significantly on par. They showed the mean 1000 seed weight of 27.55g, 27.44g, 27.43g, 27.42g, 27.41g, 27.40g, 27.39g, 27.31g, 27.30g, 27.21g, 27.20g, 27.10g, 27.02g, 27.01g and 27.00 respectively. The control recorded the mean 1000 seed weight of 21.00 g.

The effect of different treatments on yield/pot showed that the plants which were treated with the treatment, tetracycline 250 ppm (T9) recorded the maximum yield of 109.23 g/pot and were found significantly different from other treatments. The treatments *viz.*, tetracycline 100 ppm (T8) and tetracycline 50 ppm (T7) were found on par. They showed the mean yield of 96.46 g and 94.07 g/pot respectively. Treatments *viz.*, bacteria from vermicompost VB-69 (T6), endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4), rhizosphere bacteria RR-53 (T2), rhizosphere bacteria RR-26 (T1), streptocycline 250 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T16), KAU-Pf1(T20), cow dung extract 2% + vermicompost extract 2% (T15), streptocycline 200 ppm (T10) and Bactrinashak 250 ppm (T12) were found on par. They showed yield of 88.21g, 87.60g, 87.11g, 86.83g, 86.60g, 86.46g, 86.05g, 85.77g, 84.68g, 84.85g and 84.67g respectively where as the control recorded the minimum yield of 35.55g/ pot.

The effect of different on mean straw yield showed that the treatments *viz.*, rhizosphere bacteria RR-26 (T1), tetracycline 250 ppm (T9), vermicompost extract 2% + *P. fluorescens* 2% (T17), endosphere bacteria RE-1(T3), rhizosphere bacteria RR-53 (T2), bacteria from cow dung CB-39 (T4), tetracycline 50 ppm (T7), cow dung extract 2% + *P. fluorescens* 2% (T16), bacteria from vermicompost VB-69 (T6), tetracycline 100

ppm (T8) and KAU-Pf1(T20) were showed significantly higher straw yield. They showed the mean straw yield of 88.80g, 87.80g, 87.60g, 87.50g, 87.20g, 87.10g, 87.00g, 86.81g, 86.80g, 86.60g and 86.10g respectively where as the control recorded the straw yield of 50.40g only.

Owley and Windham (2003) suggested seed bacterization as one of the most successful methods of introducing biocontrol agents into an agricultural system whereby the antagonists are delivered as close to the target as possible which will protect the planting material/seed from infection by soil borne pathogens. Furthermore, foliar sprays of antagonists and incorporation into soil are also common approaches to biological control against target pathogen. Effectiveness of seed treatment and soil drenching of the rhizobacterial isolates were observed by many workers (Karuna *et al.*, 1997 and Akbar, 2002). Bhowmik *et al.* (2002) also observed that seed bacterization with the endophyte isolate Endo PR8 protected the cotyledonary infection caused by *Xanthomonas campestris* pv.*malvacearum* in cotton. Manav and Thind (2002) evaluated two rhizobacterial antagonists *viz.*, *B.subtilis* and *P. fluorescens* in pot experiments as seed treatment, seedling dip and foliar sprays and found significant reduction of bacterial blight disease intensity. According to Jigang *et al.* (2005) a novel rhizoplane diazotrophic plant growth promoting bacterium (PGPB) *Delftia tsuruhatensis* strain HR4, from the rice in North China when applied as seed treatment and foliar spray could reduce the disease incidence by 30 per cent and 24 per cent respectively in the cultivar Nonghu 6. Pupakdeepan and Prathuangwong (2010) also found that the rhizosphere bacterial strain X46 (powder formulation), by seed treatment and two foliar sprays resulted in 71.60 per cent decrease in bacterial blight severity in the treated plants under greenhouse study. Sreekumar and Nair (1990) reported that under pot culture conditions, cow dung extract was better than the chemical treatments like terramycin, streptocycline and Bactrinol-100.

### 5.10. Compatibility of bacterial antagonists with agrochemicals against *Xoo*

While adopting integrated disease management practices using antagonists, it is necessary that the agrochemicals used in the fields should be compatible with the

biocontrol agents and further care must be taken to select suitable combinations. With this in view, laboratory experiments were carried out to study the compatibility of bacterial antagonists with agrochemicals against *Xoo*.

Seven antagonistic bacteria *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67 and Pf1 were subjected to compatibility studies against *Xoo* under *in vitro* by paper disc method. 21 types of two way combinations were studied for their compatibility against *Xoo*. Two combinations *viz.*, VB-67 with VB-69, VB-67 with CB-39 showed non compatible effect in inhibiting the *Xoo*. The rest of the 17 combinations showed synergistic effect in inhibiting the *Xoo*. Ramamoorthy *et al.* (2001) opined that, management of multiple pathogens and pests in crop plants can be achieved by applying mixture of strains showing synergistic action.

The compatibility of six antagonists *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67 and VB-69 along with the reference culture Pf1 were studied with nine pesticides *viz.*, chlorpyriphos, dimethoate, quinalphos, dichlorvos, triazophos, carbendazim, mancozeb, propiconazole and hexaconazole and with four fertilizers *viz.*, urea, rajphos, muriate of potash and ammonium sulphate under *in vitro* against *Xoo*. The study showed that, out of 91 two way combinations tried, 12 combinations *viz.*, RE-1 with dimethoate, VB-69 with dichlorvos, RR-53 with triazophos, Pf1 with carbendazim, VB-69 with hexaconazole, RR-53 with hexaconazole, RR-26 with rajphos, RR-26 with muriate of potash, RR-26 with ammonium sulphate, CB-39 with ammonium sulphate and RR-53 with rajphos showed non compatible effect against *Xoo* and the rest showed synergestic effect against *Xoo*. The compatability of *P. fluorescens* with carbendazim is in agreement with the result of Vidhyasekaran and Muthamilan (1996) and compatability of *P. fluorescens* with mancozeb is in agreement with the works of Mathew(2003), Bhavani (2004) and Paul (2004). Kumar *et al.* (2011) also reported the compatibility of *Bacillus subtilis* MBI 600 (commercial formulation, Integral) with carbendazim and azoxystrobin up to 400 ppm. The compatibility of *P. fluorescens* with quinalphos and chlorpyriphos is in agreement with the results of Mathew (2003) and Paul (2004). Bhavani (2004) observed that the fertilizers *viz.*, rajphos and muriate of potash were compatible with the antagonists, where

as urea, ammonium chloride and ammonium sulphate showed varying levels of inhibition, indicating their partial compatibility.

The compatibility of 13 agrochemicals *viz.*, chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole, hexaconazole, urea, rajphos, muriate of potash and ammonium sulphate with nine pesticides *viz.*, chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole and hexaconazole was studied under *in vitro* against *Xoo*. The study revealed that out of 71 two way combinations tested, 19 combinations *viz.*, chlorpyriphos with carbendazim, chlorpyriphos with urea, dimethoate with muriate of potash, dimethoate with ammonium sulphate, triazophos with propiconazloe, triazophos with urea, triazophos with muriate of potash, quinalphos with hexaconazole, quinalphos with urea, quinalphos with muriate of potash, dichlorvos with carbendazim, carbendazim with propiconazloe, carbendazim with hexaconazole, carbendazim with urea, carbendazim with rajphos, carbendazim with muriate of potash, carbendazim with ammonium sulphate, hexaconazole with urea and hexaconazole with rajphos showed non compatible effect against *Xoo*. Three two way combinations *viz.*, Quinalphos with dichlorvos, dichlorvos with urea and hexaconazole with ammonium sulphate showed compatible action against *Xoo* and the rest 50 two way combinations showed synergistic action in inhibiting the pathogen. Manav and Thind (2001) also studied the *in vitro* efficacy of five chemicals against *Xoo* by paper disc diffusion method and found that copper sulphate and monocrotophos could inhibit the bacterial growth.

The compatibility of four fertilizers *viz.*, urea, rajphos, muriate of potash and ammonium sulphate showed that, out of six two way combinations, only one namely urea with rajphos showed non compatible action in inhibiting the *Xoo* and the rest five two way combinations showed synergistic action in inhibiting the pathogen.

All the seven antagonistic bacteria and 17 agrochemicals showed the compatible reaction in inhibiting the *Xoo*.

## 5.11. Mode of action of promising bacterial isolates

Based on the preliminary screening under *in planta* experiment, six promising bacterial antagonists obtained from rice rhizosphere, rice endosphere, cow dung and vermicompost including the reference culture Pf1, showing promising effect in increasing growth and yield of rice were subjected to various analyses for studying the attributes that contribute growth promoting effect. Consequently, they were tested for the production of IAA, hydrogen cyanide, ammonia and also their effect on phosphorous solubilization in comparison with the reference culture *P. fluorescens*.

### 5.11.1. Production of hydrogen cyanide

All the six selected bacterial isolates were tested for their ability to produce hydrogen cyanide (HCN) and observed that all were cyanogenic in nature where as Pf1 was not cyanogenic. The production HCN by the fluorescent pseudomonads were reported by Defago *et al.* (1990) and reported that the *P. fluorescens* strain CHA0 could produce HCN. Similarly the *P. fluorescens* CRb-17 strain effective in managing the *Xanthomonas axonopodis malvacearum* was the most effective producer of HCN (Mondal *et al.*, 2000). Inability to produce HCN by rhizobacterial isolates have been noticed by other workers also (YongHoon *et al.*, 2001 and Samanta and Dutta, 2004). The production of HCN by rhizobacterial strains obtained from various crops was also reported by many workers (Datta *et al.*, 2010; Rana *et al.*, 2011; Karimi *et al.*, 2012; Niranjana and Hariprasad., 2012 and Kumar *et al.*, 2012). According to them multifunctional PGPR may be used for improvement of crop performance.

### 5.11.2. Production of ammonia

The ability of the rhizobacterial isolates for the production of ammonia, a volatile compound having direct bearing on biocontrol activity were tested and it was found that all the six isolates and Pf-1 produced more ammonia. Production of ammonia by rhizobacteria from mustard has been documented by Samanta and Dutta (2004) and

concluded that ammonia production has a role in suppressing *S.sclerotiorum.* Further, Ryu *et al.* (2003) opined that volatiles produced by PGPR strains trigger growth promotion and ISR in *Arabidopsis thaliana.* Kumar *et al.* (2012) also found that three French bean rhizosbacteria obtained from Shimla and Solan were found positive for ammonia production.

### 5.11.3. Phosphorus (P) solubilization

It is well established that one of the important criteria for an efficient PGPR is their ability to transform unavailable 'P' to the available form. Thus, in the present study, the phosphorous solubilization capacity of the selected bacterial isolates was tested in Pikovaskya's TCP agar as well as in its broth. Of the six bacterial isolates and reference cultures of KAU-(Pf1), all produced phosphate solubilization zones on Pikovaskya's TCP medium plates. The zone of 'P 'solubilization on agar plates ranged from 3.5 to 6 mm in a week. RR-26 showed the maximum diameter of clear zone of 6 mm followed by CB-39 (5.87). The isolate RE-1, VB-69 and the Pf1 produced zone of 5.5 mm. Broth assay also proved the efficacy of the isolates in solubilization of phosphorus which ranged from 2.67 to 5 mg/50 ml of the culture media, the maximum being with RE-1, VB-69 and Pf1. Such capacity of rhizobacterial isolates in solubilizing 'P' were documented by many workers (Katiyar and Goel, 2003; Dey *et al.,* 2004). Further, conferring resistance of plants to stress conditions by mobilizing 'P' for plant growth was also reported. The rhizobacterial strains from different crops solubilizing phosphorus was also reported by previous workers (Cakmakcj *et al.*, 2007; Djuric *et al.*, 2011 and Shankarrao, 2012). They suggested that the rhizosphere soil is the rich source of phosphate solubilizing bacteria that can promote the plant growth by more than one PGPR trait.

### 5.11.4. Assay of growth promoting hormones

It is well established that many rhizobacteria including fluorescent pseudomonads produce plant growth promoting substances like gibberellins, cytokinins and IAA which can directly or indirectly modulate plant growth and development. Growth regulators like auxins and gibberellins are known to be produced by rhizobacteria

in many phytosysytems which has a direct effect on growth and development of plants (Patten and Glick, 1996). Hence, in this study, the capability of six rhizobacterial isolates in IAA production was assessed. All the isolates produced varying levels of IAA ranging from 15.02 to 20.32 $\mu gml^{-1}$.The maximum amount of IAA was produced by CB-39. The role of plant growth regulators has been well demonstrated by many researchers throughout the globe on a plethora of crops with different genera by beneficial bacteria (Suslow, 1982; Patten and Glick, 2002; Khalid *et al.*, 2004; Ebtsam *et al.*, 2005). Production of IAA by different PGPR strains was quantified by many workers (Glick, 1995; Rubio *et al.*, 2000; Bano and Mussarat, 2003; Bhatia *et al.*, 2005). They also noticed variation among the rhizobacterial strains in the production of IAA. The production of IAA by rhizobacterial strains was also reported by the previous workers (Khakipour *et al.*, 2008; Ashrafuzzaman *et al.*, 2009; Lavakush *et al.*, 2012 and Mishra and Kumar, 2012). They opined that when bacterial strains were used as bioinoculum, there was significant increase in plants growth.

**5.11.5. Detection of siderophores**

After assessing the various parameters which are known to contribute to plant growth, an effort was made to detect the production of secondary metabolite like siderophores by the rhizobacterial isolates as these secondary metabolites play a significant role in imparting resistance and disease suppression.

Iron is a growth-limiting factor for majority of organisms. In many cases, unavailability of iron results in deleterious effects in the growth of organisms. In order to overcome this situation, certain bacteria have built-in mechanisms, which under iron-limiting conditions, selectively chelate iron for their own purpose and make it unavailable to others. This is true with the antagonistic bacteria which produce a metabolite, siderophore the production of which is correlated with antagonistic potential. Hence, the potential bacterial antagonists selected in this study were tested for their capacity to produce siderophores.

All the islates could produce siderophore.VB-69 produced a zone of colouration of 26 mm. The other cultures produced a zone of colouration from 15 to 25 mm. This implies their low competitiveness with the iron uptake mechanisms and may explain the weak antibiosis of these isolates. Similar findings of siderophore production by CAS assay were reported by Reddy *et al.* (2004) and Storey (2005). Maleki *et al.* (2010) also found that the cucumber PGPR strain *P. fluorescens* (CV6) was shown to have broad spectrum *in vitro* antagonistic activity against *Phytophthora drechsleri*, causal agent of root rot of cucumber and the bacteria could produce considerable amount of siderophore and indole-3-acetic acid (IAA). Soltani *et al.* (2012) found five isolates of fluorescent pseudomonads obtained from wheat rhizosphere that could produce siderophore on Chrome Azurol S (CAS) agar plates.

### 5.11.7. Production of non-volatile metabolites

Production of non volatile metabolites was studied with cellophane method. The maximum inhibition was recorded by RE-1(50) and VB-69 (50) followed by RR-26 (45) and Pf1(45). The minimum per cent inhibition was recorded in RR-53 (25), CB-39 (25) and VB-67(25).Bacon and Hinton (2003) reported that, endophytic bacteria *Bacillus mojavansis* could produce some inhibitory substances into medium that was responsible for the inhibition of the hyphae of the fungus *Phytophthora*. Gangwar and Sinha (2010) reported that the *P. fluorescens* strains *viz.*, Pf 83, FLP84 and FLP 27 could produce non volatile substances and was responsible for the inhibition of *Xoo*.

## 5.12. Field experiment for the management of bacterial blight disease of rice

Based on the *in planta* evaluation and compatibility studies, the best 17 treatments which included the bactericides, organics, antagonists and bacterial consortia were tested for their efficacy in controlling bacterial blight disease under field condition.

### 5.12.1. Effect of antagonists, organics and bactericides for the management of bacterial blight disease under field condition

Per cent disease incidence showed that 16 treatments were found significantly effective in managing the bacterial blight disease than the control. Among the various

treatments, the plots which received tetracycline 100 ppm (T12) ranked first with PDI value of 43.33 followed by tetracycline 50 ppm (T11- 46.66) and were found to be highly effective in managing the disease. The plots which received endosphere bacteria RE-1(T3), bacteria from cow dung CB-39 (T4), bacteria from vermicompost VB-69 (T5), bacterial consortium (RE-1+CB-39), bacterial consortium (CB-39+VB-69) and streptocycline 250 ppm (T9) ranked third with PDI value of 50.The plots treated with Bactrinashak 250 ppm (T10) showed the PDI value of 53.33. All the above nine treatments were found on par with each other in checking the disease. The absolute control (T17) recorded the maximum PDI value of 83.33.

Per cent disease severity showed that the 16 treatments were found significantly effective in managing the disease than the control. Among the various treatments, the plots which received tetracycline 100 ppm (T12) ranked first with PDS value of 15.08 followed by tetracycline 50 ppm (T11- 17.11) and bacterial consortium (RE-1 + CB-39) (T6-17.83) were found to be significantly effective in managing the disease. The treatments *viz.*, bacterial consortium (T7- CB-39+VB-69), bacteria from cow dung (T4-CB-39), cow dung extract 2% + KAU-(Pf1) 2% (T15), endosphere bacteria (T3-RE-1), bacteria from vermicompost (T5-VB-69) and rhizosphere bacteria (T1-RR-26) were found next in managing the disease with the PDS values of 18.42, 18.54, 18.63, 18.72, 18.73 and 19.19 respectively. The control (T17) recorded the maximum PDS value of 49.32.

Coefficient of infection showed that all the treatments were found to be significantly effective in managing the disease than the control. Among the various treatments, tetracycline 100 ppm (T12) and tetracycline 50 ppm (T11) were found significantly superior in managing the disease with the CI values of 6.52 and 7.98 respectively. The treatments *viz.*, bacterial consortium RE-1+CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1 (T3), bacteria from vermicompost VB-69 (T5), streptocycline 250 ppm (T9), Bactrinashak 250 ppm (T10), cow dung extract 2% + KAU-(Pf1) 2% (T15), rhizosphere bacteria RR-26 (T1) and KAU- (Pf1) 2% (T16) were stood next in managing

the disease with the CI values of 8.91, 9.21, 9.29, 9.36, 9.37, 9.85, 10.46, 10.52, 10.86 and 11.91 respectively. The control (T17) recorded the maximum CI value of 41.11.

### 5.12.2. Effect of antagonists, organics and agrochemicals on biometric characters

The effect of different treatments on plant height, number of tillers were studied at 60 DAT where as the yield attributes and the root length and root weight were studied at the time of harvest.

The treatments *viz.*, bacterial consortium RE-1+ CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1 (T3), rhizosphere bacteria RR-26 (T1) and bacteria from vermicompost VB-69 (T5) were found superior in increasing the plant height. They showed the mean plant height of 86.33 cm, 86.16 cm, 85.73 cm, 84.93 cm, 84.06 cm and 83.60 cm respectively. The treatments *viz.*, cow dung extract 2% + KAU-(Pf1) 2% (T15), KAU-(Pf1) 2% (T16), cow dung extract 2% + vermicompost extract 2% (T14) and Bactrinashak 250 ppm (T10) were found next in increasing the plant height. They showed the mean plant length of 82.40cm, 82.06 cm, 81.73cm and 81.36 cm respectively. The treatments *viz.*, streptocycline 250 ppm (T9), tetracycline 100 ppm (T12), rhizosphere bacteria RR-53 (T2) and tetracycline 50 ppm (T11) were found on par with the mean plant height of 79.60 cm, 79.51 cm, 79.43 and 79.10 cm respectively. The control (T17) recorded the mean shoot length of 72.93 cm.

The treatments *viz.*, tetracycline 100 ppm (T12), tetracycline 50 ppm (T11) and bacterial consortium RE-1 + CB-39 (T6) were found significant in increasing the number of tillers with the mean tiller numbers of 11.43, 11.13 and 11 respectively. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4) and endosphere bacteria RE-1 (T3) were found next in increasing the tiller numbers with the mean tiller numbers of 10.91,10.83 and 10.53 respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + KAU-(Pf1) 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14) and streptocycline 250 ppm (T9)

were found on par with the mean tiller numbers of 10.43, 10.26, 10.19 and 10.08 respectively. The treatments *viz.*, KAU-(Pf1) 2% (T16), bacteria from vermicompost VB-69 (T5) and Bactrinashak 250 ppm (T10) were found on par with the mean tiller numbers of 10.03, 10 and 9.91 respectively. The treatment rhizosphere bacteria RR-53 (T2) was found next with the mean tiller number of 8.80. The treatments *viz.*, streptocycline 50 ppm (T8), cow dung extract 2% (T13) and absolute control (T17) were found least in increasing the tiller number. They recorded the mean tiller number of 7.90, 7.86 and 7.53 respectively.

The effect of different treatments on root length was studied at the time of harvest of the crop. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7) and bacterial consortium RE-1 + CB-39 (T6) were found superior in increasing the root length with the root length of 22.62 cm and 22.57 cm respectively. The treatments *viz.*, cow dung extract 2% + KAU-(Pf1) 2% (T15), KAU-(Pf1) 2% (T16), endosphere bacteria RE-1(T3), bacteria from vermicompost VB-69 (T5) and rhizosphere bacteria RR-26 (T1) were found next in increasing the root length with the root length of 19.10 cm, 19 cm, 19 cm, 18.90 cm and 18.90. The treatments *viz.*, cow dung extract 2% + vermicompost extract 2% (T14) and rhizosphere bacteria RR-53 (T2) were found on par with the mean root length of 18.03 cm and 17.90 cm respectively. The treatment Bactrinashak 250 ppm (T10) was found next with the mean root length of 17.59 cm. The treatments *viz.*, tetracycline 100 ppm (T12) and tetracycline 50 ppm (T11) were found on par. They showed the mean root length of 17.20 cm and 17.05 cm respectively. The treatment streptocycline 250 ppm was found next with the mean root length of 16.80 cm. The treatments *viz.*, cow dung extract 2% (T13) and streptocycline 50 ppm (T8) were found on par with the mean root length of 16.10 cm and 16.00 cm respectively. The absolute control (T17) recorded the minimum mean root length of 14.80 cm.

The effect of different treatments on root weight was recorded after drying the root samples. The treatments *viz.*, bacterial consortium CB-39+VB-69(T7) and bacterial consortium RE-1 + CB-39 (T6) were found significantly superior in increasing the root weight with the mean root weight of 17.53 g and 17.50 g and respectively. The treatments

*viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + KAU-(Pf1) 2% (T15) and bacteria from vermicompost VB-69 (T5) were found next in increasing the root weight with the mean root weight of 15.50 g, 15.20 g and 15.10 g respectively. The treatments *viz.*, endosphere bacteria RE-1 (T3), bacteria from cow dung CB-39 (T4) and cow dung extract 2% + vermicompost extract 2% (T14) were found on par. They showed the mean root weight of 15 g, 14.90 g and 14.80 g respectively. The treatments *viz.*, KAU-(Pf1) 2% (T16) and rhizosphere bacteria RR-53 (T2) were found on par with the mean root weight of 14.20 g and 14 g respectively. The treatments *viz.*, Bactrinashak 250 ppm (T10), cow dung extract 2% (T13), streptocycline 50 ppm (T8) and tetracycline 100 ppm (T12) were found next with the mean root weight of 12.06 g, 11.82 g, 11.80 g and 11.80 g respectively. The treatments *viz.*, tetracycline 50 ppm (T11) and streptocycline 250 ppm (T9) were found on par with the mean root weight of 11.51g and 11.50 g respectively. The absolute control (T17) recorded the minimum mean root weight of 8.50 g.

The effect of different treatments on the mean panicle length showed that the treatments *viz.*, tetracycline 100 ppm (T12), tetracycline 50 ppm (T11), bacterial consortium RE-1 + CB-39(T6), bacteria from cow dung CB-39(T4) and endosphere bacteria RE-1(T3) were found significantly effective in increasing the panicle length with the mean panicle length of 20.86cm, 20.82cm, 20.80cm, 20.50cm and 20.16cm respectively. The treatments *viz.*, bacterial consortium CB-39+VB-69 (T7), streptocycline 250 ppm (T9), bacteria from vermicompost VB-69 (T5), rhizosphere bacteria RR-26 (T1), Bactrinashak 250 ppm (T10), KAU-(Pf1) 2% (T16), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14), and streptocycline 50 ppm (T8) were found next in increasing the panicle length. They showed the mean panicle length of 19.86cm, 19.54cm, 19.53cm, 19.50cm, 19.49cm, 19.40cm, 19.26cm, 19.10cm and 18.93cm respectively. The treatments *viz.*, cow dung extract 2% (T13) and rhizosphere bacteria RR-53 (T2) were found next with the mean panicle length of 18.76cm and 18.13 respectively. The absolute control (T17) showed the mean panicle length of 16.70 cm.

The effect of different treatments on the number of filled grains/panicle showed that the plots which received the treatments *viz.*, tetracycline 100 ppm (T12) and

tetracycline 50 ppm (T10) were found significantly superior in increasing the number of filled grains/panicle. They showed the mean filled grain of 102.66 and 100 respectively. The treatments *viz.*, bacterial consortium RE-1 + CB-39 (T6), cow dung extract 2% + KAU-(Pf1) 2% (T15), tetracycline 50 ppm (T11), bacteria from vermicompost VB-69 (T5) and cow dung extract 2% + vermicompost extract 2% (T14) were found next with the number of filled grains of 99.33, 99.33, 99, 98.66 and 97 respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), streptocycline 250 ppm (T9), bacteria from cow dung CB-39 (T4) and Bactrinashak 250 ppm (T10) were found on par. They showed the mean number of filled grains of 94, 93.66, 92.33 and 92 respectively. The treatments *viz.*, endosphere bacteria RE-1 (T3) and rhizosphere bacteria RR-53 (T2) were found on par with the mean number of filled grains of 90.66 and 88.66 respectively. The treatments *viz.*, cow dung extract 2% (T13) and KAU-(Pf1) 2% (T16) were found on par with the mean number of filled grains of 86.33 and 85.66 respectively. The treatment streptocycline 50 ppm (T8) recorded the mean number of filled grains of 77. The absolute control recorded the minimum number of filled grains of 66.66.

The effect of different treatments on the 1000 seed weight showed that the plots which received the treatments *viz.*, tetracycline 100 ppm (T12), tetracycline 100 ppm (T11), bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1(T3), streptocycline 250 ppm (T9), bacteria from vermicompost VB-69 (T5) and Bactrinashak 250 ppm (T10) were found significantly superior in increasing the 1000 seed weight with the mean 1000 seed weight of 28.30g, 28.20g, 28.10g, 27.88g, 27.80g, 27.60g, 27.60g, 27.40g and 27.30g respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), KAU-(Pf1) 2% (T16), cow dung extract 2% + *P. fluorescens* 2% (T15) and cow dung extract 2% + vermicompost extract 2% (T14) were found next in increasing the 1000 seed weight with the mean 1000 seed weight of 26.80g, 26.40g, 25.80g and 25.50 g respectively. The treatment rhizosphere bacteria RR-53 (T2) was found next with the mean 1000 seed weight of 25.20 g. The treatments *viz.*, streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par. They showed the mean 1000 seed weight of 24.80g and

24.80g respectively. The absolute control (T17) recorded the minimum mean 1000 seed weight of 23.20g.

The plots which received the treatment tetracycline 100 ppm (T12) recorded significantly higher yield of 3261.00 kg/ha. The treatments *viz.*, tetracycline 50 ppm (T11), bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7) and bacteria from cow dung CB-39 (T4) were found next in increasing the yield. They recorded the yield of 2949.89, 2928.47, 2904.65 and 2899.89 kg/ha respectively. The treatments *viz.*, endosphere bacteria RE-1(T3), bacteria from vermicompost VB-69 (T5), streptocycline 250 ppm (T9) and Bactrinashak 250 ppm (T10) were found on par with the yield of 2857.04, 2849.75, 2842.75, and 2797.52 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14) and KAU-(Pf1) 2% (T16) were found on par with the yield of 2749.90, 2700.11, 2696.66 and 2664.18 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-53(T2), streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par with the yield of 2557.03, 2500, 2485.62 kg/ha respectively. The absolute control (T17) recorded the lowest yield of 1821 kg/ha.

The plots which received the treatments *viz.*, tetracycline 100 ppm (T12), bacterial consortium RE-1 + CB-39 (T6), bacterial consortium CB-39+VB-69 (T7), bacteria from cow dung CB-39 (T4), endosphere bacteria RE-1(T3) and bacteria from vermicompost VB-69 (T5) were found significantly superior over other treatments in increasing the straw yield. They showed the mean straw yield of 3534.00, 3520.00, 3501.43, 3487.76, 3435.28 and 3415.86 kg/ha respectively. The treatments *viz.*, rhizosphere bacteria RR-26 (T1), tetracycline 50 ppm (T11), cow dung extract 2% + *P. fluorescens* 2% (T15), cow dung extract 2% + vermicompost extract 2% (T14), streptocycline 250 ppm (T9) and KAU-(Pf-1) 2% (T16) were found on par with the mean straw yield of 3314.28, 3251.68, 3246.30, 3240.00, 3235.98 and 3193.93 kg/ha respectively. The treatments *viz.*, Bactrinashak 250 ppm (T10), streptocycline 50 ppm (T8) and cow dung extract 2% (T13) were found on par with the mean straw yield of 3083.60, 3005.00 and 2990.66

kg/ha respectively. The absolute control (T17) recorded the minimum straw yield of 2193.33 kg/ha.

The application of cow dung for the management of bacterial leaf blight was in agreement with the previous results (Mary *et al.*, 1986; Das *et al.*, 1998 and Sible *et al.*, 2004).The application of *P. fluorescens* as seed treatment, soil application and foliar spray was found in minimizing the bacterial leaf blight of rice and recorded higher yield was also in agreement with the previous reports (Nayar and Vidhyasekaran, 1998; Vidhyasekaran *et al.*, 2001; Manav and Thind 2002; Velusamy and Gnanamanickam, 2003 and Jayalakshmi *et al.*, 2010). Mathew and Anitha (2012) also observed the effectiveness of combined application of cow dung slurry (suspension) with 2% *P.fluorescens* for the management of bacterial blight of rice.

Summing up the results of the study discussed so far, the bacterial blight pathogen from Palakkad district are highly virulent than that of Alappuzha and Thrissur districts. During the survey, two phases of the bacterial blight *viz.*, kresek and leaf blight were observed. The kresek symptom appeared as pale yellow leaves initially followed by rolling along the midrib, withering, wilting and drying of young plants, where as leaf blight symptoms during flowering stage, appeared as wavy yellow lesions on both sides of the leaves leaving a small green area in the centre. Finally the lesions become papery white and leaves were dried. On artificial inoculation also the same types of symptoms were obtained. The infected plants collected from 14 locations on isolation could yield circular, convex, yellow and slimy colonies on PSA medium. The pathogen from different locations differed in cultural, morphological and biochemical characters. The pathotype study evidenced the high virulence of pathogens from Palakkad district. The DNA figerprinting corroborated the high variability among the 14 isolates of the pathogen.

Among the bactericides, tetracycline (50,100 and 250 ppm), streptocycline (200 and 250 ppm) and Bactrinashak 250 ppm were effective in managing the highly virulent isolates obtained from Athimani, Nenmara, Parali and Polpully areas of Palakkad district

under *in vitro* condition. Among the organics and agrochemicals tested under *in vitro,* cow dung extract 2% + vermicompost extract 2%, cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% +*P. fluorescens* 2%, vermicompost extract 2 % and copper hydroxide (0.15% ) were found effective. The six bacterial isolates *viz.,* RE-1, RR-26, CB-39, RR-53 VB-67 and VB-69 were effective under *in vitro* against the pathogen. Based on the cultural, morphological and biochemical characters, these isolates were identified as *Pseudomonas* sp.

The pot culture experiment, proved that tetracycline (50,100,250ppm), streptocycline 250 ppm, Bactrinashak 250 ppm, bacteria from cow dung CB-39, bacteria from vermicompost VB-69, endosphere bacteria RE-1, rhizosphere bacteria RR-26, cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2% and cow dung extract 2% + vermicompost extract 2%, rhizosphere bacteria RR-53 and copper hydroxide (0.15%) were found best in that order, in managing the bacterial blight disease of rice.

Compatibility studies among seven antagonistic bacteria *viz.,* RE-1, RR-26, RR-53, CB-39, VB-67 along Pf1(reference culture) were studies against *Xoo* under *in vitro.* The 17 combinations showed synergistic effect in inhibiting the *Xoo.* The compatibility of seven antagonists with nine pesticides revealed that 71 two way combinations showed synergistic effect against *Xoo.* The compatibility of 13 agrochemicals under *in vitro* against *Xoo,* revealed that 50 two way combinations showed synergistic action in inhibiting the pathogen. Three two way combinations showed compatible action in inhibiting the pathogen. The compatibility of four fertilizers *viz.,* urea, rajphos, muriate of potash and ammonium sulphate showed that five two way combinations showed synergistic action in inhibiting the pathogen. All the seven antagonistic bacteria and 17 agrochemicals showed the compatible reaction in inhibiting the *Xoo.*

Six promising bacterial antagonists obtained from rice rhizosphere, rice endosphere, cow dung and vermicompost showing promising effect disease suppression and in increasing growth and yield of rice under *in planta* were subjected to various mode

of action studies *viz.*, production of siderophore, IAA, hydrogen cyanide, ammonia and also their effect on phosphorous solubilization in comparison with the reference culture *P. fluorescens*. All the isolates could produce siderophore and IAA. A few isolates could produce non volatile metabolites. The field study confirmed that tetracycline (50 and 100 ppm), bacterial consortium (RE-1+CB-39), bacterial consortium (CB-39+VB-69), bacteria from cow dung (CB-39), endosphere bacteria (RE-1), bacteria from vermicompost (VB-69), streptocycline 250 ppm, Bactrinashak 250 ppm, cow dung extract 2% + KAU-(Pf1) 2%, rhizosphere bacteria (RR-26) and KAU-(Pf1) 2% are promising in managing the bacterial blight disease of rice.

# *Summary*

## 6. SUMMARY

Bacterial blight of rice is considered as one of the major constraints of rice cultivation in Kerala, as it causes severe loss to the crop. The seed and soil borne nature of the pathogen and its wide host range on weed hosts makes the management of the disease less effective. Considering the serious nature of the disease, the present investigation on "Pathogenic and genetic variability in *Xanthomonas oryzae* pv.*oryzae* (*Xoo*) (Ishiyama) Swings *et al.* and management of bacterial blight disease" was carried out at Regional Agricultural Research Station (RARS), Pattambi, Kerala, India. The study was mainly intended on analyzing the various aspects of the disease particularly the cultural, morphological, biochemical, pathogenic and genetic variability of the pathogen, *in vitro* sensitivity of bactericides; mechanism of action of bacterial antagonists; studies on the compatibility of bacterial antagonists with common agrochemicals against *Xoo*, the bacterial blight pathogen; and effective management of the pathogen using bactericides, biocontrol agents, different organics and agrochemicals. The salient findings of the present study are summarized below:

6.1. A survey was conducted during September 2007 in 14 locations *viz.*, Athimani, Erattakulam, Kodallur, Manchira, Nenmara, Parali, Pattambi, Polpully, Akamala, Kodakara, Mannuthy, Edathua, Karuvatta and Moncombu of three major rice growing districts of Kerala *viz.*, Alappuzha, Palakkad and Thrissur. The bacterial blight disease incidence was recorded in two farmer preferred popular varieties namely Uma (MO-16) and Jyothi (PTB-39) revealed that the pathogen from the five locations namely Erattakulam, Athimani, Parali, Polpully and Nenmara in Palakkad district recorded susceptible reaction indicating the existence of highly virulent pathogen population *Xoo* in the district.

6.2. In the surveyed rice fields, water soaked lesions which turn yellow in colour on the leaf margins from tip to bottom were observed initially. As the disease progressed, the characteristic symptom of bacterial blight, as long wavy stripes covering the entire leaf margin, leaving the small green portion in the centre was also observed.

6.3. The infected samples collected from the 14 locations, on isolation on PSA medium yielded typical yellow, slimy, convex and circular colonies. Pathogenicity test on the susceptible variety Jyothi (PTB-39), could give the typical symptom of bacterial blight, and on re isolation, yielded the colonies of the original culture.

6.4. Cultural and morphological studies established that there were not much variation among the isolates. The observed differences were in the colony shape, colour, sliminess and in time taken for colony appearance. Out of the 14 isolates, eight isolates *viz.,* Athimani (XAMI-1), Erattakulam (XERM-5), Kodallur (XKOR-3), Manchira (XMRA-4), Parali (XPAI-5), Akamala (XAKA-4), Mannuthy (XMTY-2) and Karuvatta (XKVA-2) showed 1 to 2 mm diameter colonies where as the remaining six isolates *viz.*, Nenmara (XNRA-5), Pattambi (XPTB-2), Polpully (XPLY-3), Kodakkara (XKDA-3), Edathua (XEDA-3) and Moncombu (XMOU-2) showed colonies of 1 to 3 mm diameter. Only two colonies differed from the normal shape of circular and convex colonies. Moncombu (XMOU-2) and Manchira (XMRA-4) isolates had irregular margin of colonies. Two isolates *viz.,* Akamala (XAKA-4) and Edathua (XEDA-3) showed light yellow colonies and slightly differed from dark yellow colonies of the rest. Two isolates *viz.,* Akamala (XAKA-4) and Edathua (XEDA-3) had moderate slimy colonies where as the other twelve isolates showed good sliminess. The isolates *viz.,* Athimani (XAMI-1) and Polpully (XPLY-3) took 36 h for colony appearance whereas Erattakulam (XERM-5), Nenmara (XNRA-5) and Parali (XPAI-5) isolates took 48 h. The rest took only 24 h.

6.5. Fourteen isolates showed positive for Gram's reaction, starch hydrolysis, levan formation, arginine dihydrolase and 3% KOH solubility test. They differed in three biochemical tests *viz.,* citrate utilization, gelatin liquefaction and $H_2S$ production. Parali (XPAI-5) and Pattambi (XPTB-2) isolates could utilize citrate. Except Kodallur (XKOR-3) and Nenmara (XNRA-5) isolates, all the other 12 isolates could liquefy gelatin. $H_2S$ was produced by Parali (XPAI-5) isolate alone. The isolates slightly differed in acid production from 14 different carbohydrates indicating their variability. Based on the cultural, morphological and biochemical studies, the pathogen causing bacterial blight disease was tentatively identified as *X. oryzae* pv. *oryzae* (Ishiyama) Swings *et al.*

6.6. Pathotype study was conducted in the net house using 14 isolates on 20 commonly cultivated rice varieties and six near isogenis lines having different R genes/gene combination and two rice differentials with no genes could distinguish the virulence spectrum of the isolates into three groups/categories namely highly virulent, moderately virulent and weakly virulent isolates/strains. The five isolates from Palakkad district *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Nenmara (XNRA-1), Parali (XPAI-3) and Polpully (XPLY-5) came under the first group of 'highly virulent', the seven isolates *viz.*, Akamala (XAKA-2), Mannuthy (XMTY-2), Edathua (XEDA-3), Karuvatta (XKVA-1), Kodakara (XKDA-1), Manchira (XMRA-2) and Pattambi (X PTB-4) came under the second group of 'moderately virulent' and the rest two isolates *viz.*, Kodallur (XKOR-3) and Moncombu (XMOU-1) came under the third group of 'weakly virulent' isolates/strains.

6.7. Genetic variability of 14 isolates was studied using the BOX and ERIC-PCR fingerprinting. The dendrogram showed the existence of high level of genetic variability among the pathogen population in the rice growing areas of Kerala. The isolates *viz.*, Manchira (XMRA-2), Polpully (XPLY-5) and Moncombu (XMOU-1) clustered tightly showing more than 90 per cent similarity. Similarly, the isolates *viz.*, Pattambi (X PTB-4) and Edathua (XEDA-3) showed more similarity (94%) and were tightly clustered. The remaining isolates *viz.*, Athimani (XAMI-3), Erattakulam (XERM-1), Kodallur (XKOR-3), Nenmara (XNRA-1), Parali (XPAI-3), Akamala (XAKA-2), Kodakara (XKDA-1), Mannuthy (XMTY-2) and Karuvatta (XKVA-1) showed very high variability (less than 80% similarity) within themselves and other isolates each other.

6.8. *In vitro* sensitivity of six bactericides with three concentrations *viz.*, 50,100 and 250 ppm against four highly virulent isolates of *Xoo* revealed that tetracycline 250 ppm was found to be the best antibiotic followed by tetracycline 100 ppm and tetracycline 50 ppm. This was closely followed by streptocycline 250 ppm, streptocycline 200 ppm and Bactrinashak 250 ppm.

6.9. *In vitro* sensitivity of different organics and agrochemicals tested against the highly virulent Polpully isolate of the *Xoo* revealed that cow dung extract 2% + vermicompost extract 2% showed maximum per cent inhibition of 47 which was found on par with cow dung extract 2% + *P. fluorescens* 2% (45.83). Vermicompost extract 2% + *P. fluorescens* 2% ranked next with per cent inhibition of 43.75.Vermicompost extract 2 % alone found next with per cent inhibition of 37.08 and was on par with copper hydroxide 0.15% (33.75). Cow dung extract showed per cent inhibition of 29.16. This gives a possibility for the management of bacterial blight by organics.

6.10. Isolation and enumeration of total microflora from rice rhizosphere, rice endosphere, cow dung and vermicompost revealed more abundance of bacteria in rice rhizosphere, rice endosphere and in two organic sources. As high as 110 bacterial isolates obtained from this study screened against the highly virulent isolate of *Xoo* from Polpully could yield six prominent bacterial isolates *viz.*, endosphere bacteria (RE-1) from Kodallur, rhizosphere bacteria from Nenmara (RR-26), rhizosphere bacteria from Pattambi (RR-53), cow dung bacteria from Pattambi (CB-39) and vermicompost bacterial isolates (VB-67 and VB-69) from Pattambi based on maximum antagonism index values.

6.11. Cultural and morphological characteristics of promising bacterial isolates on nutrient agar medium showed that the bacterial isolates *viz.*,RE-1, RR-26, CB-39 and RR-53 showed 2 to 3 mm, white and circular colonies, whereas VB-67 and VB-69 produced 2 to 3 mm, white to light brown, circular colonies. Biochemical characterization showed that all of them were found positive for Gram's reaction, starch hydrolysis, gelatin liquefaction, levan formation, 3% KOH solubility, tryptophan utilization, arginine dihydrolyse and catalase tests. The HIMEDIA KB 002 HiAssorted$^{TM}$ Biochemical Test Kit reaction showed that little variation existed among them indicating that they were different from each other. Based on cultural, morphological and biochemical studies, the antagonists were tentatively identified as *Pseudomonas* sp.

6.12. The pot culture experiment conducted with 21 treatments consisted of selected antagonists, bactericides, fungicides and two organics. Results showed that

tetracycline 250 ppm, tetracycline 100 ppm, tetracycline 50 ppm, streptocycline 250 ppm, Bactrinashak 250 ppm, bacteria from cow dung (CB-39), bacteria from vermicompost (VB-69), endosphere bacteria (RE-1), rhizosphere bacteria (RR-26), cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2% and cow dung extract 2% + vermicompost extract 2%, rhizosphere bacteria (RR-53) and copper hydroxide 0.15% were found best in managing the bacterial blight disease.

6.13. Seven bacterial antagonistic bacteria *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67, VB-69 and Pf1 were subjected to compatibility studies against *Xoo* under *in vitro*. The study revealed that except the two combinations *viz.*, VB-67 with VB-69 and VB-67 with CB-39, the rest of the 17 combinations showed synergistic effect in inhibiting *Xoo*.

6.14. The compatibility of seven antagonists were tested with nine pesticides *viz.*, chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos,carbendazim, mancozeb, propiconazole and hexaconazole and with four fertilizers *viz.*, urea, rajphos, muriate of potash and ammonium sulphate under *in vitro* against *Xoo*. Out of 91 two way combinations tried, 71 two way combinations were found having synergestic effect against *Xoo* and the rest 12 combinations *viz.*, RE-1 with dimethoate, VB-69 with dichlorvos, RR-53 with triazophos, Pf1 with carbendazim , VB-69 with hexaconazole, RR-53 with hexaconazole, RR-26 with rajphos, RR-26 with muriate of potash, RR-26 with ammonium sulphate, CB-39 with ammonium sulphate and RR-53 with rajphos showed non compatible effect against *Xoo*.

6.15. The compatibility of thirteen agrochemicals *viz.*, chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole, hexaconazole, urea, rajphos, muriate of potash and ammonium sulphate with nine pesticides *viz.*, chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole and hexaconazole under *in vitro* against *Xoo*. The study revealed that out of 71 two way combinations tested, 50 two way combinations showed synergistic action in inhibiting the pathogen. Three two way combinations *viz.*, quinalphos with dichlorvos, dichlorvos with urea and hexaconazole with ammonium

sulphate showed compatible action against *Xoo* and the rest 19 combinations *viz.*, chlorpyriphos with carbendazim, chlorpyriphos with urea, dimethoate with muriate of potash, dimethoate with ammonium sulphate, triazophos with propiconazloe, triazophos with urea, triazophos with muriate of potash, quinalphos with hexaconazole, quinalphos with urea, quinalphos with muriate of potash, dichlorvos with carbendazim, carbendazim with propiconazloe, carbendazim with hexaconazole, carbendazim with urea, carbendazim with rajphos, carbendazim with muriate of potash, carbendazim with ammonium sulphate, hexaconazole with urea and hexaconazole with rajphos showed non compatible effect against *Xoo.*

6.16. The compatibility of four fertilizers *viz.*, urea, rajphos, muriate of potash and ammonium sulphate showed that out of six two way combinations, only one namely urea with rajphos showed non compatible action in inhibiting the *Xoo* and the rest five two way combinations showed synergistic action in inhibiting the pathogen. All the seven antagonistic bacteria and 17 agrochemicals showed compatible reaction in inhibiting the *Xoo.*

6.17. The seven promising bacterial antagonists were subjected for various growth promoting characters *viz.*, 'P' solubilization, $NH_3$ and HCN production. The six isolates differed slightly for the above characters.

6.18. All the isolates could produce siderophore. VB-69 produced a zone of colouration of 26 mm. The other cultures produced a zone of colouration from 15 to 25 mm. All the isolates produced varying levels of IAA ranging from 15.02 to 20.32 $\mu gml^{-1}$. The maximum amount of IAA was produced by CB-39 (20.32 $\mu gml^{-1}$). The remaining isolates produced comparatively high quantity of IAA which ranged from 15.02 to 20.32 $\mu gml^{-1}$.

6.19. Production of non volatile metabolites by the promising antagonists in comparison with reference culture Pf1 was tested by the cellophane method. At three days after inoculation, the per cent inhibition of the pathogen varied from 25 to 50.The

maximum inhibition was recorded by RE-1 (50) and VB-69 (50) followed by RR-26 (45) and Pf1(45).The minimum per cent inhibition was recorded in RR-53 (25), CB-39 (25) and VB -67 (25).

6.20. The field studies of the present investigation have come out with a new array of management practices for combating bacterial blight disease in Kerala. The promising treatments to manage the disease were tetracycline 100 ppm, tetracycline 50 ppm, bacterial consortium (RE-1+CB-39), bacterial consortium (CB-39+VB-69), bacteria from cow dung (CB-39), endosphere bacteria (RE-1), bacteria from vermicompost (VB-69), streptocycline 250 ppm, Bactrinashak 250 ppm, cow dung extract 2% + KAU-(Pf1) 2%, rhizosphere bacteria RR-26 and KAU-(Pf1) 2%, cow dung extract 2% + vermicompost extract 2%, and rhizosphere bacteria (RR-53).

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# Appendices

## APPENDIX 1

# MEDIA COMPOSITION

(Ingredients per litre)

Composition of different media used for various studies

**Potato Sucrose Agar (PSA)**

| | | |
|---|---|---|
| Potato | : | 300 g |
| $Ca(No_3)$ 4 $H_2o$ | : | 0.5 g |
| $Na_2HPo_4$ | : | 2 g |
| Peptone | : | 5 g |
| Sucrose | : | 20 g |
| Agar | : | 15 g |

**Martin's rose bengal streptomycin agar medium (MRBA)**

| | | |
|---|---|---|
| Dextrose | : | 10.0 g |
| Peptone | : | 5.0 g |
| $KH_2PO_4$ | : | 1.0 g |
| $MgSO_4$ | : | 0.5 g |
| Agar | : | 20.0 g |
| Rose Bengal | : | 0.03 g |
| Streptomycin | : | 30.0 mg (added aseptically) |
| Distilled water | : | 1000 ml |

**Thornton's agar media (TT) (pH 7.4)**

| | | |
|---|---|---|
| Mannitol | : | 1.0 g |
| Asparagine | : | 0.5 g |
| $K_2HPO_4$ | : | 1.0 g |
| $KNO_3$ | : | 0.5 g |
| $MgSO_4$ | : | 0.2 g |
| $CaCl_2$ | : | 0.1 g |
| NaCl | : | 0.1g |
| $FeCl_3$ | : | 0.002 |
| Agar | : | 20.0 g |
| Distilled water | : | 1000 ml |

**Kenknights agar medium (KK) (pH 7.0)**

| | | |
|---|---|---|
| Dextrose | : | 1.0 g |
| $KH_2PO_4$ | : | 0.1 g |
| $NaNO_3$ | : | 0.1 g |
| KCl | : | 0.1g |
| $MgSO_4$ | : | 0.1g |
| Agar | : | 20.0 g |
| Distilled water | : | 1000 ml |

**Nutrient Agar Medium (NA)**

| | | |
|---|---|---|
| Peptone | : | 5.0g |
| Beef extract | : | 1.0g |
| Sodium Chloride | : | 5.0g |
| Agar agar | : | 20.0g |
| pH | : | 6.5 to 7. |

**King's B Medium**

| | |
|---|---|
| Peptone | : 20.0 g |
| Glycerol | : 10.0 ml |
| $K_2HPO_4$ | : 10.0 g |
| $MgSO_4.7H_2O$ | : 1.5 g |
| Agar agar | : 20.0 g |
| pH | : 7.2 – 7.4 |

**Luria Bertani Medium (LB medium)**

| | |
|---|---|
| Tryptone | : 10.0 g |
| Yeast extract | : 5. 0 g |
| NaCl | : 5. 0 g |
| Glucose | : 1. 0 g |
| pH | : 7.0 |

**Simmon's Citrate Agar**

| | |
|---|---|
| Ammonium dihydrogen phosphate | : 1g |
| Dipotassium phosphate | : 1g |
| NaCl | : 5g |
| Sodium Citrate | : 2g |
| Magnesium Sulphate | : 0.2 g |
| Agar agar | :15g |
| Bromothymol blue | : 0.08g |

**Peptone water (pH 7.0)**

| | |
|---|---|
| Peptone | : 10.0 g |
| NaCl | : 15.0 g |
| Distilled water | : 1000 ml |

**Pikovaskya's medium (pH 7.0)**

| | |
|---|---|
| Glucose | : 10.0 g |
| $Ca(PO_4)_3$ | : 5.0 g |
| $NH_4SO_4$ | : 0.5g |
| KCl | : 0.23g |
| $MgSO_4$ | : 0.1g |
| $MnSO_4$ | : trace |
| $FeSO_4$ | :trace |
| Yeast extract | :0.5g |
| Agar | :20.0 g |
| Distilled water | : 1000ml |

**Methyl red agar media (MRA) (pH 7.0)**

| | |
|---|---|
| Beef extract | : 3.0 g |
| Peptone | : 5.0 g |
| Methyl red | : 150 mg |
| Distilled water | : 1000 ml |

Dissolve 150mg methyl red in 10ml water and sterilize separately and add after autoclaving the medium.

**Christensen's urea agar medium**

| | |
|---|---|
| Peptone | : 1 g |
| Nacl | : 5 g |
| $KH_2PO4$ | : 2 g |
| Glucose | : 10 g |
| Agar | : 20 g |

**MRVP – broth**

| | |
|---|---|
| Peptone | : 7g |
| Dextrose | : 5 g |
| K2HPO4 | : 5 g |
| Distilled water | : 1 L |
| pH | : 6.9 |

**Methyl red (MR) test**

| | |
|---|---|
| Methyl red | : 1 g |
| Ethanol | : 300 ml |
| Distilled water | : 200 ml |

**Barrtis –A**

| | |
|---|---|
| α naphthol | : 5 g |
| Ethanol | : 95 ml |

**Barrtis – B**

| | |
|---|---|
| KOH | : 40 g |
| Creatine | : 0.3 g |
| Distilled water | : 100 ml |

**Nutrient gelatin medium**

| | |
|---|---|
| Peptone | :10 g |
| Beef extract | :5 g |
| Gelatin | : 120 g |
| Distilled Water | :1000 ml |
| pH | :7.0 |

**Tryptic soy agar**

| | |
|---|---|
| Soybean casein enzymic hydrolysate | : 15.0g |
| Papaic digest of soybean meal | : 5.0g |
| Sodium chloride | : 5.0g |
| Agar | : 15.0g |
| Distilled water | : 1000ml |
| pH | : 7.0-7.2 |

**Crystal violet agar**

| | |
|---|---|
| Agar | : 15g |
| Lactose | : 10g |
| Peptone | : 5g |
| Beef extract | : 3g |
| Crystal violet | : 0.0033g |
| Distilled water | : 1000ml |
| pH | : 6.8 |

## Fiss Minimal Medium

### Composition of modified Fiss Minimal Medium for assay of siderophores

The following stock solutions were prepared, autoclaved ($FeSO_4$ was steam sterilized) and stored at 4°C.

a. **Potassium phosphate ($KH_2PO_4$) and Asparagine solution:** 0.524 per cent solution of $KH_2PO_4$ and L-Asparagine were prepared by dissolving 5 g of $KH_2PO_4$ and 5 g of L-Asparagine in Millipore water to make a final volume of 954 ml. The pH was adjusted to 6.8 with a solution of 6.0 M NaOH.

b. **Glucose solution:** 50 per cent solution of glucose was prepared by dissolving 50 g of glucose in Millipore water to make a final volume of 100 ml.

c. **Manganese sulphate ($MnSO_4$) solution:** 0.001 per cent solution of $MnSO_4$ was prepared by dissolving 0.001 g $MnSO_4$ in Millipore water to make a final volume of 100 ml.

d. **Magnesium sulphate ($MgSO_4$) solution:** 0.4 per cent solution of $MgSO_4$ was prepared by dissolving 0.4 g $MgSO_4$ in Millipore water to make a final volume of 100 ml.

e. **Zinc chloride ($ZnCl_2$) solution:** 0.005 per cent solution of $ZnCl_2$ was prepared by dissolving 0.005 g $ZnCl_2$ in Millipore water to make a final volume of 100 ml.

f. ***Ferrous sulphate ($FeSO_47H_2O$) solution:*** *1mM solution of* $FeSO_4$ *was prepared* by dissolving 0.0278 g $FeSO_4.7H_2O$ in Milli pore water to make a final volume of 100 ml. The solution was subjected to tyndallisation.

Modified Fiss minimal medium contained: 5.03 $gl^{-1}$ $KH_2PO_4$, 5.03 $gl^{-1}$ Asparagine, 5.0 $gl^{-1}$ glucose, 40 $mgl^{-1}$ $MgSO_4$, 100 $\mu gl^{-1}$ $MnSO_4$, and 500 $\mu gl^{-1}$ $ZnCl_2$. Iron-restricted modified Fiss minimal medium was prepared by adding 139 $\mu gl^{-1}$ $FeSO_4$ to the final medium (0.5 $\mu M$). High iron modified Fiss minimal medium was prepared by adding 5.56 $mgl^{-1}$ $FeSO_4$ to the final medium (20 $\mu M$).

## APPENDIX II

### Agarose Gel Electrophoresis for DNA studies

**Solution I: pH 8.0 (100 ml)**

| | | |
|---|---|---|
| Glucose 50 mM | : | 901.0 mg |
| Tris HCl 25 mM | : | 303.0 mg |
| EDTA 100 mM | : | 336.0 mg |

To 80 ml dd $H_2O$ add salts, dissolve and make up to 100 ml adjust pH to 8.0.

**Solution II**

| | | |
|---|---|---|
| 2 M NaOH | : | 0.1 ml |
| 10.0 % SDS | : | 0.1 ml |
| St. $H_2O$ | : | 0.8 ml |
| Total | : | 1.0 ml |

It should be prepared freshly each time.

**TE buffer pH: 8.0 (100 ml)**

1 M Tris pH- 8.0 was prepared by dissolving 12.1 gm base in 80 ml of water. pH adjusted to 8.0, made up to 100 ml and autoclaved. 0.5 M EDTA pH 8.0 was prepared and autoclaved before use.

| | | | |
|---|---|---|---|
| Tris HCl (1M) | pH8.0 | : | 1.0 ml |
| EDTA (0.5 M) | pH8.0 | : | 0.2 ml |
| dd $H_2O$ | | : | 98.8 ml |

**CTAB Solution**

Dissolve 4.1 gram NaCl in 80 ml water and slowly add 10 g CTAB (Cetyl-trimethyl ammonium bromide, also called hexa decyl-trimethyl ammonium bromide) while heating and stirring. Adjust the final volume to 100 ml.

**10X TAE buffer**

| | | |
|---|---|---|
| Tris - HCl | : | 100 mM |
| EDTA | : | 10 mM |
| Sodium chloride | : | 20 mM |

The components were dissolved in 60 ml of sterile distilled water and adjusted to pH 7.4. The final volume was then made upto 100 ml with sterile distilled water, autoclaved and stored at 4°C.

**5X TBE (pH 8.0)**

| | | |
|---|---|---|
| Tris base | : | 54.0 g |
| Boric acid | : | 27.0 g |
| 0.5 M EDTA (pH 8) | : | 20.0 ml |
| Distilled water | : | 1000 ml |

The solution was autoclaved and stored at room temperature

**Tracking dye (10X)**

| | | |
|---|---|---|
| 5X TBE | : | 5.0 ml |
| Glycerol | : | 35.0 ml |
| 0.5 M EDTA | : | 2.0 ml |
| 20 per cent SDS | : | 0.50 ml |
| 10 per cent Bromophenol blue | : | 3.0 ml |

The volume was made to 50 ml with double distilled water and stored at 4°C

# ABSTRACT

# PATHOGENIC AND GENETIC VARIABILITY IN *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* AND THE MANAGEMENT OF BACTERIAL BLIGHT DISEASE

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**ABSTRACT OF THE THESIS**

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## ABSTRACT

Bacterial blight of rice is a major threat in rice cultivation causing huge yield loss to the crop. Realizing the practical importance, an investigation entitled on "Pathogenic and genetic variability in *Xanthomonas oryzae* pv.*oryzae* (Ishiyama) Swings *et al.* and management of bacterial blight disease" was carried out during 2006-2011. A series of surveys conducted in 14 locations of three major rice growing districts (Alappuzha, Palakkad and Thrissur) of Kerala during September 2007 to find out the occurrence of bacterial blight disease. High incidence was recorded in Palakkad district. During, the survey, the characteristic symptom of bacterial blight as yellow lesions on the both the margins of the leaf leaving a green area in the centre of leaf were observed. The pathogen causing bacterial blight of rice was isolated from 14 locations of the said districts and their pathogenicity was established. Based on the cultural, morphological and biochemical characters, the pathogen was identified as *Xanthomonas oryzae* pv.*oryzae* (*Xoo*) (Ishiyama) Swings *et al.* The 14 isolates showed slight variation in their cultural, morphological and biochemical characters.

Pathotype studies were conducted in net house using 14 isolates on 20 popular and commonly cultivated rice varieties, six near isogenic lines having different R genes/gene combination and two rice differentials with no genes. It could distinguish the virulence spectrum of the isolates into three groups/categories namely 'highly virulent', 'moderately virulent' and 'weakly virulent' isolates/strains. The study on the kresek symptom also confirmed the above finding. BOX and ERIC-PCR fingerprinting depicted the existence of high level of genetic variability among the pathogen population in the rice growing areas of Kerala.

*In vitro* sensitivity of six bactericides against the four highly virulent isolates of *Xoo* revealed that tetracycline 250 ppm, tetracycline 100 ppm, tetracycline 50 ppm, streptocycline 250 ppm, streptocycline 200 ppm and Bactrinashak 250 ppm were effective against the pathogen. *In vitro* sensitivity of different organics and agrochemicals

revealed that cow dung extract 2% + vermicompost extract 2%, cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2%, vermicompost extract 2 %, copper hydroxide 0.15% were found effective against the pathogen, in that order.

The 110 bacterial isolates obtained from rice rhizosphere, rice endosphere and cowdung and vermicompost, screened against the Polpully virulent pathogen could yield six prominent bacterial isolates *viz.,* RE-1, RR-26, RR-53, CB-39, VB-67 and VB-69 and were tentatively identified as *Pseudomonas* sp. Pot culture experiment, showed that tetracycline 250 ppm, tetracycline 100 ppm, streptocycline 250 ppm, Bactrinashak 250 ppm, tetracycline 50 ppm, bacteria from cow dung (CB-39), bacteria from vermicompost (VB-69), endosphere bacteria (RE-1), rhizosphere bacteria (RR-26), cow dung extract 2% + *P. fluorescens* 2%, vermicompost extract 2% + *P. fluorescens* 2% and cow dung extract 2% + vermicompost extract 2%, rhizosphere bacteria (RR-53) and copper hydroxide 0.15% were found best in managing the bacterial blight disease.

Seven bacterial antagonists *viz.*, RE-1, RR-26, RR-53, CB-39, VB-67 VB-69 and Pfl when subjected to compatibility studies against *Xoo* under *in vitro* showed 17 combinations, showing synergistic effect in inhibiting the *Xoo.* In the compatibility study of seven antagonists with nine pesticides, 71 two way combinations were found synergestic effect against *Xoo.* The compatibility of 13 agrochemicals under *in vitro* against *Xoo,* revealed that 50 two way combinations showed synergistic action in inhibiting the pathogen. Three two way combinations showed compatible action in inhibiting the pathogen. In the compatibility of four fertilizers *viz.*, urea, rajphos, muriate of potash and ammonium sulphate showed that five two way combinations proved synergistic action in inhibiting the pathogen. All the seven antagonistic bacteria and 17 agrochemicals showed the compatible reaction in inhibiting *Xoo.*

The seven bacterial antagonists were subjected for various growth promoting characters *viz.,* 'P' solubilization, $NH_3$ and HCN production. The six isolates differed slightly for the above characters. All the isolates could produce siderophore and IAA. A few isolates could produce non volatile metabolites.

The field study established the most practical finding that the tetracycline 50 ppm, tetracycline 100 ppm, bacterial consortium (RE-1+CB-39), bacterial consortium (CB-39 +VB-69), bacteria from cow dung (CB-39), endosphere bacteria (RE-1), bacteria from vermicompost (VB-69), streptocycline 250 ppm, Bactrinashak 250 ppm, cow dung extract 2% + KAU-(Pf1) 2%, rhizosphere bacteria (RR-26) and KAU-(Pf1) 2% were found promising in managing bacterial blight disease of rice. Thus, apart from bactericides, there was a variety of highly promising organic management possibilities to combat the disease.