

## Original Research Article

# Studies on the Prevalence of bacterial leaf blight of rice in Andhra Pradesh and invitro evaluation of silver and copper nanoparticles against *Xanthomonas oryzae* pv. *oryzae*

### ABSTRACT

A roving survey was conducted during *Kharif* 2023 in major rice-growing districts of Andhra Pradesh to assess the severity of bacterial leaf blight disease in rice. The percent disease index averages from 25 to 59.6% was recorded. The maximum disease severity was observed in the tillering stage. The maximum percent disease index was observed in Chinta reddy Palem village in Nellore district (59.60%), while the minimum was recorded in Denduluru village in Eluru district (25%). The disease was more severe in the BPT-5204 variety. Another investigation on the effect of nanoparticles on *Xoo*. Copper and silver nanoparticles exhibit antimicrobial activity against *Xoo*. Silver nanoparticles exhibited the highest anti-microbial activity when compared with copper nanoparticles at 0.1% and 0.2% concentrations, respectively.

**Keywords:** Bacterial leaf blight, copper nano particles, Rice, *Xoo*

### INTRODUCTION

Rice (*Oryza sativa*) is the staple food for more than half of the world's population with nearly 90% of total global production and consumption contributing immensely to food and nutritional security, urging essentiality for sustainable rice production globally (Fukagawa and Ziska, 2019).

Bacterial leaf blight (BLB) is one of the most destructive diseases of rice in Asia (Mew, 1987). BLB infection leads to yield losses of 20%–70% depending on the variety and severity of the infection, especially in hybrid rice, the stage of the crop, and climatic conditions (Chukwu *et al.*, 2019). The losses may be even 80%–100% in cases of bacterial infection occurring at the tillering stage of the crop (Srinivasan and Gnanamanickam, 2005; Khan *et al.*, 2012). In India, this disease is prevalent in almost all paddy-growing regions and is a major problem in the *Kharif* season (Laha *et al.*, 2009).

Persistent monocropping, over fertilizer application, irrigation techniques and the widespread cultivation of susceptible cultivars by farmers have led to the development of increased resistance to pathogen, resulting in a severe outbreak of bacterial leaf blight disease in rice. In order to evaluate the prevalence of bacterial leaf blight, a survey was carried out in major rice-growing districts of Andhra Pradesh and to further develop efficient management strategies.

Nanotechnology is one of the promising strategies that finds its application in agricultural sector in the area of crop protection. Bio synthesized silver nanoparticles (AgNPs) has been found to be effective in managing the damage caused by the bacterial leaf blight in rice (Ahmed *et al.*, 2020). Recent studies have also demonstrated the extensive antimicrobial activities of silver nanoparticles (AgNPs) against bacteria due to its multiple modes of inhibitory action (Mikhailova, 2020). Copper has also been found to have potential anti-bacterial properties. Copper nanoparticles (CuNPs) have recently been reported as an economical alternative to other metal nanoparticles (Chawla *et al.*, 2020).

Anobservational studies were conducted to evaluate the potential of copper and silver nanoparticles as antibacterial agents for managing bacterial leaf blight in rice.

## MATERIALS AND METHODS

### 2.1 Survey for bacterial leaf blight in major rice growing districts of Andhra Pradesh

A roving survey was conducted to assess the severity of bacterial leaf blight disease in the major rice growing districts of Andhra Pradesh viz, East Godavari, West Godavari, Nellore, Tirupati, NTR, Eluru, and Krishna during the *kharif* season, 2023. Other parameters such as Location (GPS coordinates), stage of the crop, percent disease index and type of symptoms were also recorded.

One-square-meter quadrants were randomly selected in each field and infected plants were counted in each quadrant. The disease severity was calculated based on a scale developed by IRRI in 2014. (Table 1.). Per cent disease index (PDI) was calculated as per following formula

$$\text{PDI} = \frac{\text{Sum of all individual disease ratings}}{\text{Total number of leaves examined}} \times \frac{100}{\text{Maximum disease scale}}$$

Table.1 IRRI Scale,2014

Scale	% leaf area diseased
1	1-5%
3	6-12%
5	13-25%
7	26-50%
9	51-100%

Infected rice leaves that are collected from rice fields at different stages of crop growth were used for isolation of pathogen. Bacterial ooze was the most noticeable sign visible on the leaf blade in the early morning. Most characteristic symptoms observed during the survey are kresek, water-soaked lesions, wavy margins and wilting.

#### 2.1.1 Isolation of *Xanthomonas oryzae* pv. *oryzae* from diseased rice leaves

Seven samples representing each district were isolated for further research purpose. Samples were rinsed in sodium hypochlorite (0.1%) for 30 seconds, then rinsed in sterilized distilled water three times (1 minute) each to remove traces of sodium hypochlorite. Later, leaf samples were cut into small leaf bits and then crushed with a glass rod in centrifuge tubes for two minutes. (Bradbury *et al.*, 1970). A loopful of bacterial ooze is taken and streaked on the plates containing Wokimoto medium and incubated at 28±2 °C for three days.

#### 2.1.2 Pathogenicity test

All seven isolates of *Xanthomonas oryzae* pv. *oryzae* were grown on wokimoto medium containing petri plates for 72 hours at 28±2 °C. The yellow, circular, smooth and mucoid colonies were harvested and suspended in nutrient broth for 24 hours (approximately 5 x 10<sup>7</sup> CFU/ml). Bacterial blight susceptible rice variety, BPT-5204 was grown in pots for a pathogenicity test. The 30-day-old rice plants were inoculated with Xoo by the leaf clipping method (Kauffman *et al.*, 1973) during evening hours of the day. The plants were covered with plastic covers to maintain humid conditions.

### 2.2 Evaluation of nanoparticles against *Xanthomonas oryzae* pv. *oryzae*

Silver and copper nanoparticles were obtained from Regional Agricultural Research Station, Tirupati were evaluated at 0.1% and 0.2% concentrations against *Xanthomonas oryzae* pv. *oryzae* by using the disc diffusion method. A highly virulent Xoo6 bacterium is used in these studies. As a positive control, streptomycin sulphate @ 500 ppm and as a negative control, distilled water were also maintained. The experiment was performed in triplicate under aseptic conditions. The petri plates were then kept in incubator at 28±2°C for 72 hours. The zone of inhibition was measured in mm with the help of a scale after 72 hours of incubation.

## RESULTS AND DISCUSSION

### 3.1 Survey for bacterial leaf blight in major rice growing districts of Andhra Pradesh

Diseased rice leaves were collected at different stages during the roving survey in *Kharif*, 2023. Percentage disease index (PDI) was calculated for each district which averaged from 25% to 59.60%. The survey data (Table. 1, Fig. 1) reveals that the highest percent disease index of 59.60 % was recorded chintha reddy palem in Nellore district, followed by Kaikaluru village in Krishna district with 47.8 %, and the least PDI of 25% was recorded in Denduluru village in Eluru district. The maximum disease severity was observed during the tillering stage. The disease was more severe in the BPT-5204 variety compared to other varieties. The major constraints for bacterial leaf blight in Andhra Pradesh might be monocropping, cultivating susceptible rice varieties, excessive nitrogen application and congenial environmental conditions like humidity and rainfall plays a major role in disseminating the pathogen to surrounding areas, resulting in moderate-to-severe epidemics (Obradovic *et al.*, 2004; Kumar *et al.*, 2020).

The results in accordance with Laha *et al.* (2024) who reported the occurrence of 70 percent incidence of bacterial leaf blight in Guntur district in BPT-5204 variety during 2013. Similarly, Laha *et al.* (2024) reported 30-80 percent incidence was observed in BPT-5204 and MTU-3626 varieties in East and West Godavari districts during the year 2014 in Andhra Pradesh.

Raghunandana *et al.* (2023) conducted random survey in rice growing ecosystem districts of Karnataka during *kharif* 2019. Among all the surveyed ecosystems, the highest mean per cent disease index of 52.60 was observed in the Bhadra ecosystem and the lowest PDI of 31.08 were observed under the Kaveri ecosystem.

#### 3.1.1 Isolation of *Xanthomonas oryzae* pv. *oryzae* isolates

All seven isolates of *Xanthomonas oryzae* pv. *oryzae* isolated (Fig. 2) on wokimoto medium resulted in yellow-coloured, mucoid, smooth, convex colonies were seen (Fig. 3) (Shankara *et al.*, 2017).

The colonies of *Xanthomonas oryzae* pv. *oryzae* isolated from the collected diseased samples exhibited yellow, circular, smooth, convex and round bacterial colonies on nutrient agar medium (NA) which were similar to the characteristics presented by (Shankara *et al.*, 2017; Kavitha *et al.*, 2020).

#### 3.1.2 Pathogenicity

After 14 days of *Xoo* inoculation, characteristic symptoms like water-soaked lesions and wavy margins along the margins of leaves. Among all isolates *Xoo* 6 exhibited severe symptoms (Fig. 4). Later, the pathogen was re-isolated and compared with the original *Xoo* culture to prove its pathogenicity.

Similar kind of observations were made by (Shankara *et al.*, 2017; Chandraprakash *et al.*, 2018) after 14 days of post-inoculation, water-soaked lesions and wavy margins were clearly recorded in rice leaves.

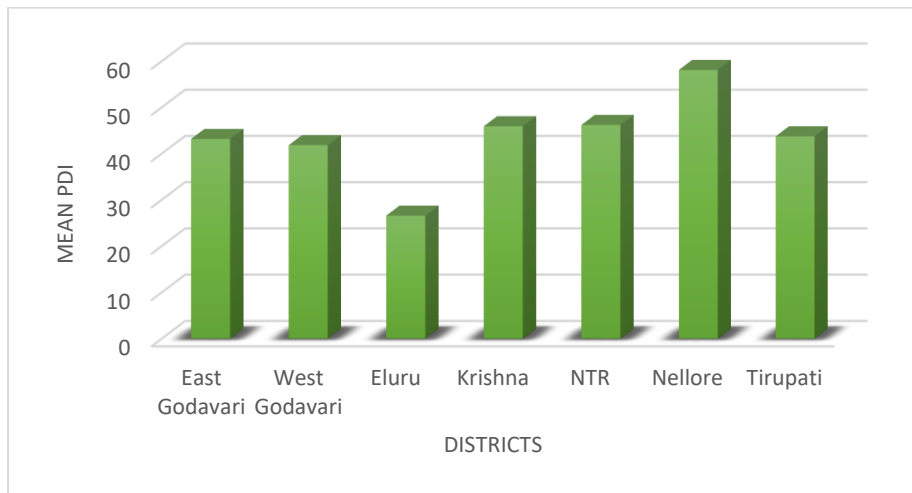
The results were similar with (Akhtar *et al.*, 2009) who studies the different methods of inoculation of BLB pathogen for pathogenicity viz., clip inoculation, pin prick inoculation, and brush methods. Among the three methods of inoculation, distinct, clear lesions were obtained in the clip inoculation method.

### 3.2 Evaluation of nanoparticles against *Xanthomonas oryzae* pv. *oryzae*

Studies revealed sensitivity of silver and copper nanoparticles were evaluated against *Xanthomonas oryzae* pv. *oryzae*. *Xoo*6 bacterial isolate is used in this study. Among the nanoparticles, silver nanoparticles showed the highest antibacterial activity at two different concentrations (0.1% and 0.2%) produced maximum inhibition zone of 15.3 mm at 0.1% and 25.6 mm at 0.2% concentration and the least inhibition zone was recorded by copper nanoparticles at 9.1 mm at 0.1 % and 15.1 mm at 0.2% concentration. The antibiotic streptomycin sulfate @500ppm showed a clear inhibition zone of 23 mm. The zone of inhibition was not observed in control. The values are significant data pertaining to zone of inhibition (mm) are depicted in Table 2. (Fig. 5).

The results are in accordance with Arnab *et al.*, (2024) reported that silver nanoparticles exhibit highest antibacterial properties and produces a maximum inhibition zone of 13mm when compared to copper nanoparticles which exhibits lowest antibacterial properties produces a inhibition zone of 8mm against *Xanthomonas oryzae* pv. *oryzae*.

According to studies given by Le thi *et al.*, (2021) silver nanoparticles with a concentration of more than 5 µg/mL exhibited a strong antibacterial effect against strain of *Xanthomonas oryzae* pv. *oryzae* VXO\_281.



**Fig. 1. Disease severity of BLB of rice in major rice growing districts during in Andhra Pradesh kharif, 2023**

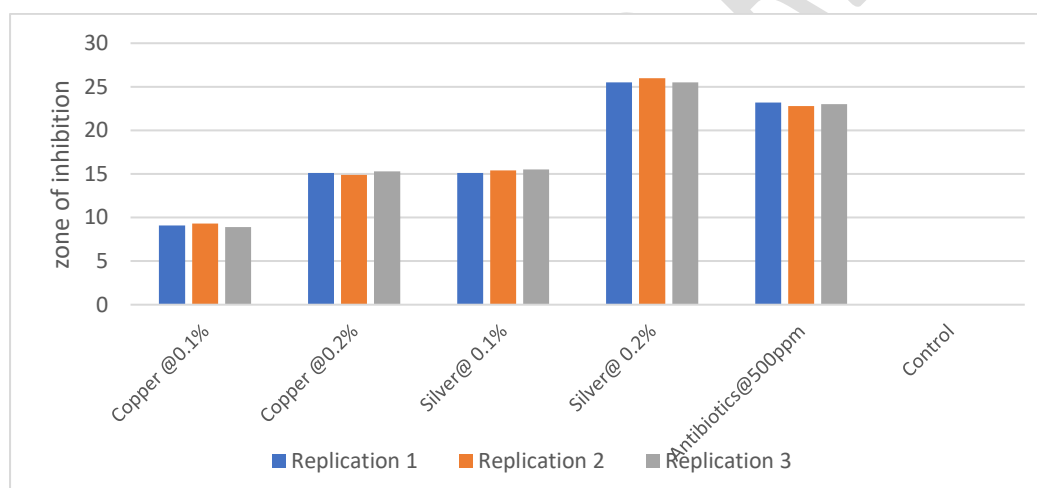
**Table.2. Percent disease index of bacterial leaf blight disease during *kharif* 2023 in major rice growing districts of Andhra Pradesh**

District	Villages	Location	Stage of the crop	Variety Of the crop	Type of symptoms	Laboratory name of isolate	Per cent Disease index (PDI)	Mean per cent Disease index (PDI)
East Godavari	Rajahmahendravaram Peravalli kovvur	N16 <sup>0</sup> .668 E81 <sup>0</sup> .780 N 16 <sup>0</sup> .752 E 81 <sup>0</sup> .7416 N17 <sup>0</sup> .0188 E 81 <sup>0</sup> .7303	Transplanting stage	MTU-1318 PLA-1100 BPT-5204	Kresek , Wilting	Xoo 1	43.67 43.5 42.28	43.15
West Godavari	Maruteru Dubacherla Nallajerla	N 16 <sup>0</sup> .37'52.9 E 81 <sup>0</sup> 44'48.8 N 16 <sup>0</sup> .917 E 81 <sup>0</sup> .350 N 16 <sup>0</sup> .947115 E 81 <sup>0</sup> .405359	Seedling stage	BPT-5204 TN1 MTU-2077	Water-soaked lesions, wavy margins	Xoo 2	40.72 41.22 43.5	41.81
Eluru	Kalaparru Denduluru Bhimadole	N 16 <sup>0</sup> .67 E 81 <sup>0</sup> .00 N 16 <sup>0</sup> .7609 E 81 <sup>0</sup> .1665 N 16 <sup>0</sup> .8108 E 81 <sup>0</sup> .2637	Vegetative stage	MTU-1121 BPT-5204 MTU-1318	Kresek	Xoo 3	26.20 25.00 28.5	26.56
Krishna	Avanigadda Gannavaram kaikaluru	N 17 <sup>0</sup> .1'22.26 E 80 <sup>0</sup> .42'571 N 16 <sup>0</sup> .5419 E 80 <sup>0</sup> .8050 N16 <sup>0</sup> .541 E 81 <sup>0</sup> .202	Transplanting stage	MTU-1010 MTU-5204 PLA-1100	Water-soaked lesions	Xoo 4	45.5 44.6 47.8	45.9
NTR	Tiruvuru Nandigama Jaggayyapeta	N 16 <sup>0</sup> 24'49.49 E 80 <sup>0</sup> 47'914 N 16 <sup>0</sup> .7724 E 80 <sup>0</sup> .2859 N 16 <sup>0</sup> .8985 E 80 <sup>0</sup> .1033	Vegetative stage	MTU-1061 BPT-5204 MTU-1010	Water-soaked lesions	Xoo 5	46.27 45.0 44.3	46.19
Nellore	Balayapalli Chinthareddy Palem Muthukur	N 13 <sup>0</sup> 58'42.2E 79 <sup>0</sup> 36'13.3 N 14 <sup>0</sup> .4426 E 79 <sup>0</sup> .9865 N14 <sup>0</sup> .226 E 80 <sup>0</sup> .1000	Tillering stage	BPT-5204 BPT-5204 BPT-5204	Water-soaked lesions, wavy margins	Xoo 6	58.20 59.60 56.7	58.1
Tirupati	Perumalpalli Chandragiri Naravari palli	N 13 <sup>0</sup> .568 <sup>0</sup> E 79 <sup>0</sup> .40 <sup>0</sup> N 13 <sup>0</sup> .5880 E 79 <sup>0</sup> .3159 N13 <sup>0</sup> .6188 E 79 <sup>0</sup> .2663	Tillering stage	NLR-999 BPT-5204 RNR-3228	Wavy margins	Xoo 7	43.56 41.8 45.8	43.72

**Table 3. Efficacy of antibiotics against *Xanthomonas oryzae* pv. *oryzae***

S. No.	Nano particles	Mean
1.	Copper @0.1%	9.1 <sup>d</sup>
2.	Copper @0.2%	15.1 <sup>c</sup>
3.	Silver@ 0.1%	15.33 <sup>c</sup>
4.	Silver@ 0.2%	25.67 <sup>a</sup>
5.	Antibiotics@500ppm	23 <sup>b</sup>
6.	Control	0 <sup>e</sup>
	C.V	1.26
	C.D at 5%	0.40
	SEm±	0.05
	P	.00

\*Different letters in superscript indicate significant difference ( $P < 0.05$ ). Out of all, treatments 4 is significantly differs from other treatments followed by treatments 5, treatment 2 and 3 are on par with each other, followed by treatment 1. There is no inhibition zone observed in treatment 6.



**Fig. 2. zone of inhibition (mm) formed by nanoparticles at 0.1% and 0.2 %concentrations**



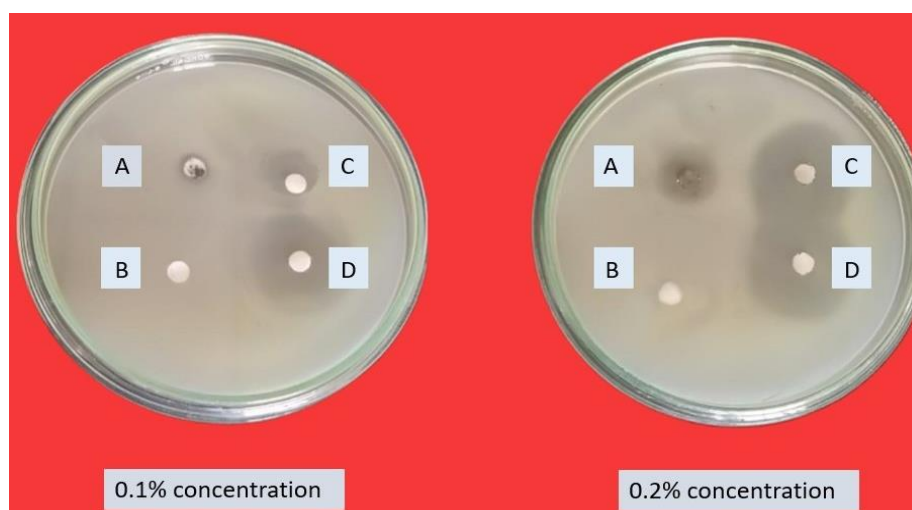
**Fig.3. Symptoms of bacterial leaf blight in different stages of rice crop**



**Fig.4. characteristic colonies of Xoo**



**Fig.5. Pathogenicity test for *Xanthomonas oryzae* pv. *oryzae***



**Fig.6. Inhibition zone produced by nano particles against *Xanthomonas oryzae* pv. *oryzae*.** A. depicts zone of inhibition formed by copper nanoparticles B. zone of inhibition zone formed by control C. zone of inhibition formed by silver nanoparticles D. zone of inhibition formed by streptomycin sulfate.

### References :

- Ahmed T, Shahid M, Noman M, Niazi MBK, Mahmood F, Manzoor I, et al. Silver nanoparticles synthesized by using *Bacillus cereus* SZT1 ameliorated the damage of bacterial leaf blight pathogen in rice. *Pathogens*.2020; 9(3): 160.
- Akhtar MA, Abbasi FM, Ahmad H, Shahzad M, Shah MA ,Shah AH et al . Evaluation of rice germplasm against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight. *Pakistan Journal of Botany* .2009; 43(6): 3021-3023.
- Arnab Roy Chowdhury AR, Kumar R, Mahanty A, Mukherjee K, Kumar S, Tribhuvan KU et al. Inhibitory role of copper and silver nanocomposite on important bacterial and fungal pathogens in rice (*Oryza sativa*). *Scientific Reports* .2024;14(1):1779.
- Bradbury JH, Collins JG, Pylotis NA . Digestibility of proteins of the histological components of cooked and raw rice. *British journal of nutrition*.1984; 52(3):507-513.
- Chandraprakash S, Manonman, K, Revathy N, Devi E, Kokila Devi. Management of Bacterial Leaf Blight Disease of Paddy (*Oryza sativa*) Caused by *Xanthomonas oryzae* pv. *oryzae* using bacteriophages. *International journal of current Microbiology and Applied Sciences* .2018;7:3066-3080.
- Chawla P, Kumar N, Bains, A, Dhull SB, Kumar, M, Kaushik et al. Gum arabic capped copper nanoparticles: Synthesis, characterization and applications. *International journal of biological macromolecules*.2020; 146: 232-242.
- Chukwu SC, Rafii M Y, Ramlee S I, Ismail S I, Hasan M M and Oladosu Y A et al. Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Molecular Biology Reporter*. 2019; 46 :1519–1532.
- Fukagawa NK and Ziska LH. Rice: Importance for global nutrition. *Journal of nutritional science and vitaminology* .2019; 65: S2-S3.

- Kauffman HE. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease Reporter*. 1973; 57:537-541.
- Kavitha K, Nagamani P, Sudhan PM, Viswanath K and Reddy, NP. Studies on suppression of bacterial leaf blight by rice endophytic bacteria under field condition. 2020 *Oryza*. 57(1):64-69.
- Khan JA, Siddiq R, Arshad H MI, Anwar HS, Saleem K and Jamil FF et al. 2012. Chemical control of bacterial blight of rice caused by *Xanthomonas oryzae* pv. *Oryzae*. *Pakistan Journal of Phytopathology*. 2012; 24:97–100.
- Kumar A, Kumar R, Sengupta D, Das SN, Pandey MK, Bohra A et al 2020. Deployment of genetic and genomic tools toward gaining a better understanding of rice-*Xanthomonas oryzae* pv. *oryzae* interactions for development of durable bacterial blight resistant rice. *Frontiers in plant science* .2020;11:1152.
- Laha G Geographical Distribution, pathogen diversity and host plant resistance in bacterial blight of rice. 2009.
- Laha G S. Geographical Distribution, pathogen diversity and host plant resistance in bacterial blight of rice. *Journal of Mycopathological research*. 2024;62(2): 219-229.
- Le Thi, H, Nguyen TPH, Le Trong D, Vu TH, Le Thi, V, Hoang TG, et al. Synthesis of chitosan stabilized silver nanoparticles and evaluation of the in vitro antibacterial activity against *Xanthomonas oryzae* pv. *oryzae* causing Blight Disease of Rice. *VNU Journal of Science: Natural Sciences and Technology*. 2021;38(1).
- Mew, T.W. Current status and future prospects of research on bacterial blight of rice. 1987.359-382.
- Mikhailova EO. Silver nanoparticles: Mechanism of action and probable bio-application. *Journal of functional biomaterials*. 2020;11(4):84.
- Obradovic A, Jones JB, Momol MT, Balogh B, and Olson S M. Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. *Plant Disease reporter*. 2004 ;88: 736–740.
- Shankara K, Patil M B, Pramesh D, Sunkad G, Yenjerappa S T, Ibrahim M et al .Characterization of *Xanthomonas oryzae* pv. *oryzae* isolates from Rice Growing Regions of Southern India. *International Journal of Pure and Applied Biosciences*. 2017;5(4): 452-461.
- Srinivasan B and Gnanamanickam S. Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*. *Current Science*. 2005;88(8):1229–1231.
- Wilson M and Lindow S E. Interaction between the biological control agent *Pseudomonas fluorescens* A 506 and *Erwinia amylovora* in pear blossoms. *Phytopathology* .1993;83: 117- 123.