Advances in Research

Volume 25, Issue 4, Page 503-511, 2024; Article no.AIR.121938
ISSN: 2348-0394, NLM ID: 101666096

Prevalence of Bacterial Leaf Blight in Rice Across Andhra Pradesh and *In vitro* Assessment of Silver and Copper Nanoparticles against *Xanthomonas oryzae pv. oryzae*

Vineetha. G [a*], M. Pradeep [a], P.N Harathi [b] and T. Rajasekharam [c]

[a] *Department of Plant Pathology, S.V Agricultural College, ANGRAU, Tirupati, India.*
[b] *Department of Entomology, Regional Agricultural Research Station, ANGRAU, Tirupati, India.*
[c] *Department of Plant Pathology, Citrus Research Station, Petluru, Dr. YSRHU, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/air/2024/v25i41130

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121938

Original Research Article

Received: 13/06/2024
Accepted: 15/08/2024
Published: 21/08/2024

ABSTRACT

Bacterial leaf blight is one of the most destructive diseases in rice which leads to yield loss of 20-40%. This study aimed to assess the prevalence of bacterial leaf blight in rice across Andhra Pradesh. A roving survey was conducted during *Kharif* 2023 in Andhra Pradesh to assess

**Corresponding author: E-mail: gunnemedavineetha@gmail.com;*

Cite as: *G, Vineetha., M. Pradeep, P.N Harathi, and T. Rajasekharam. 2024. "Prevalence of Bacterial Leaf Blight in Rice Across Andhra Pradesh and In Vitro Assessment of Silver and Copper Nanoparticles Against Xanthomonas Oryzae Pv. Oryzae". Advances in Research 25 (4):503-11. https://doi.org/10.9734/air/2024/v25i41130.*

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. In spite chemical control, there are chances of development of resistance. There is a need for nanotechnology intervention for management of disease. This study also investigates the antimicrobial activity of copper and silver nanoparticles against Xoo by using paper disc diffusion method under invitro conditions. Silver nanoparticles exhibited the highest anti-microbial activity when compared with copper nanoparticles at 0.1% and 0.2% concentrations, respectively. Further research is needed to explore the field application of nanoparticles and their long-term effects on rice crops.

Keywords: Bacterial leaf blight; copper nano particles; rice; Xoo.

1. 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 [1].

Bacterial leaf blight (BLB) is one of the most destructive diseases of rice in Asia [2]. 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 [3]. The losses may be even 80%–100% in cases of bacterial infection occurring at the tillering stage of the crop [4, 5]. In India, this disease is prevalent in almost all paddy-growing regions and is a major problem in the *Kharif* season [6].

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 [7]. Recent studies have also demonstrated the extensive antimicrobial activities of silver nanoparticles (AgNPs) against bacteria due to its multiple modes of inhibitory action [8]. 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 [9].

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

2. 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

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

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.

Table 1. IRRI Scale, 2014

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

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. [10, 11]. 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 10^7 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 [12, 13] 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 *Xoo*6 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.

3. 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 [14, 15].

The results in accordance with Laha *et al*. [16] 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*. [16] reported30-80 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*. [17] 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) [11].

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 [11, 18].

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 [11, 19] after 14 days of post-inoculation, water-soaked lesions and wavy margins were clearly recorded in rice leaves.

The results were similar with [13] 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. X*oo6 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*., [20] reported that silver nanoparticles exhibit highest antibacterial properties and produces a maximum inhibition zone of 13mm when compared to copper nanoparticleswhich exhibits lowest antibacterial properties produces a inhibition zone of 8mm against *Xanthomonas oryzae* pv. *oryzae.*

According to studies given by Le thi *et al*., [21] 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 [22].

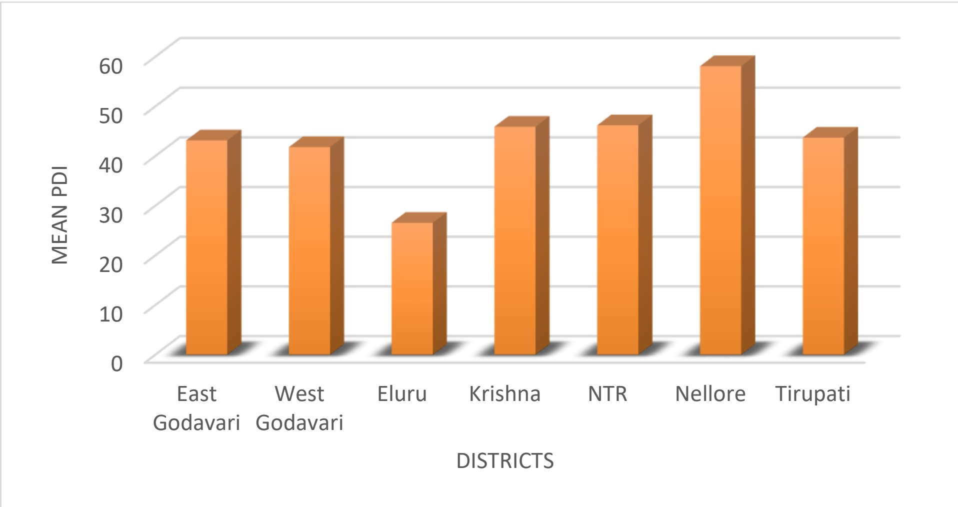

Fig. 1. Disease severity of BLB of rice in major rice growing districts during in Andhra Pradesh *kharif*, 2023

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

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

S. No.	Nano particles	Mean
1.	Copper @0.1%	9.1 [d]
2.	Copper @0.2%	15.1 [c]
3.	Silver@ 0.1%	15.33[c]
4.	Silver@ 0.2%	25.67[a]
5.	Antibiotics@500ppm	23 [b]
6.	Control	0[e]
	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.*

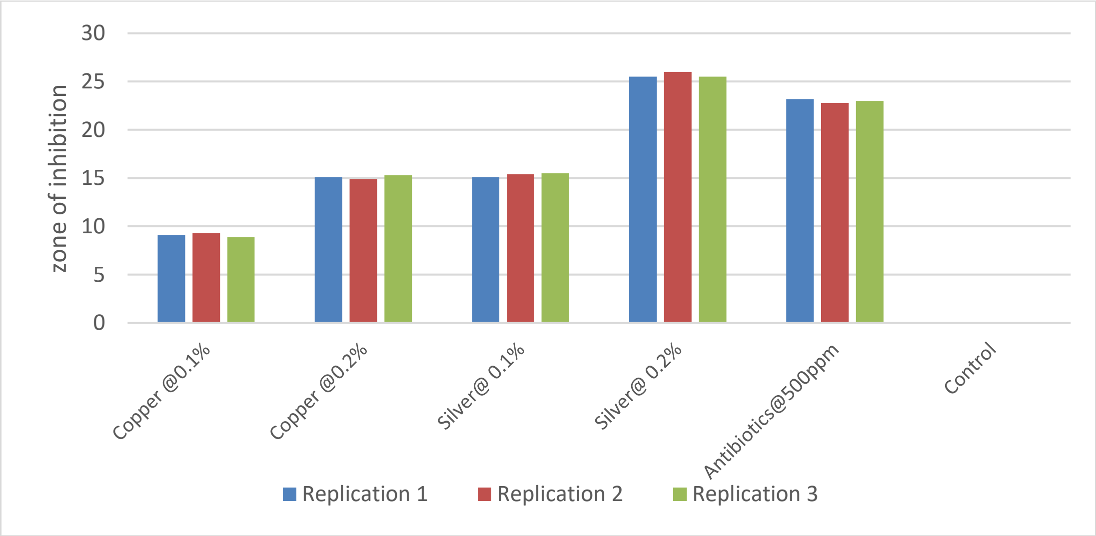

Fig. 2. Zone of inhibition (mm) formed by nanoparticles at 0.1% and 0.2 %concentrations

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*

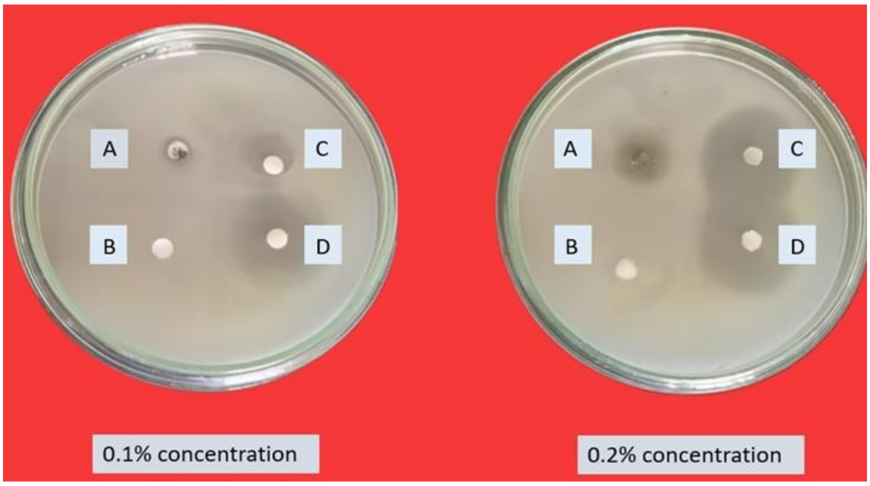

Fig. 6. Inhibition zone produced by nano particles against *Xanthomonas oryzae* pv *.oryzae*.
A. depicts zone of inhibition formed by copper nanoparticles B. zone of inhibition zone formed by control C. zone of inhibition formed by silver nanoparticles D. zone of inhibition formed by streptomycin sulfate.

4. CONCLUSION

This study demonstrates the substantial impact of bacterial leaf blight on rice cultivation in Andhra Pradesh. Disease incidence ranges from 25 to 59.6%. The severity of the disease is mainly during the tillering stage. On the other hand, there is an urgent need for effective management of the disease. While conventional methods have limitations, this study highlights the potential of nanotechnology as a promising alternative. The antimicrobial efficacy of silver and copper nanoparticles against Xanthomonas oryzae pv. oryzae offers opportunities for developing control measures. However, further research needs to be done to optimize nanoparticle formulations for field application and to assess their long-term impact on the rice ecosystem.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEGEMENT

The first author is thankful to Acharya N. G. Ranga Agricultural University, Lam, Guntur, India for providing financial support for this work

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fukagawa NK, Ziska LH. Rice: Importance for global nutrition. Journal of Nutritional Science and Vitaminology. 2019;65:S2-S3.
2. Mew TW. Current status and future prospects of research on bacterial blight of rice.1987;359-382.
3. Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Hasan MM, Oladosu YA, et al. Bacterial leaf blight resistance in rice: A review of conventional breeding to molecular approach. Molecular Biology Reporter. 2019;46:1519–1532.
4. Srinivasan B, 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.
5. Khan JA, Siddiq R, Arshad HMI, Anwar HS, Saleem K and Jamil FF, et al. Chemical control of bacterial blight of rice caused by *Xanthomonas oryzae* pv. *Oryzae.* Pakistan Journal of Phytopathology. 2012;24:97–100.
6. Laha G. Geographical Distribution, pathogen diversity and host plant resistance in bacterial blight of rice; 2009.
7. 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.
8. Mikhailova EO. Silver nanoparticles: Mechanism of action and probable bio-application. Journal of Functional Biomaterials. 2020;11(4):84.
9. 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.
10. Bradbury JH, Collins JG, Pyliotis NA. Digestibility of proteins of the histological components of cooked and raw rice. British Journal of Nutrition.1984;52(3):507-513.
11. Shankara K, Patil MB, Pramesh D, Sunkad G, Yenjerappa ST, 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.
12. Kauffman HE. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae.* Plant Disease Reporter. 1973; 57:537-541.
13. 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.
14. 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.
15. Kumar A, Kumar R, Sengupta D, Das SN, Pandey MK, Bohra A, et al. 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.

16. Laha GS. Geographical Distribution, pathogen diversity and host plant resistance in bacterial blight of rice. Journal of Mycopathological Research. 2024;62(2):219-229.
17. Raghunandana A, Pramesh D, Gururaj S, Amoghavarsha C, Yadav MK, Ngangkham U, et al. Genetic diversity and pathotype profiling of Xanthomonas oryzae pv. oryzae isolates from diverse rice growing ecosystems of Karnataka state of India. Plant Protection Science. 2023.59(1).
18. 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. *Oryza*. 2020;57(1):64-69.
19. 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.
20. 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.
21. 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).
22. Wilson M, Lindow SE. Interaction between the biological control agent *Pseudomonas fluorescens* A 506 and *Erwinia amylovora* in pear blossoms. Phytopathology. 1993;83:117- 123.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/121938