International Journal of Economic Plants

https://ojs.pphouse.org/index.php/IJEP

IJEP February 2024, 11(1): 007-011

Crossref

Article IJEP5088c

Full Research

Doi: HTTPS://DOI.ORG/10.23910/2/2024.5088c

Effect of Antagonists and Botanicals Against *Xanthomonas oryzae* pv. oryzae *In Vitro*

Bhukya Srinivas[1*], V. A. Patil[2], C. U. Shinde[3], Priya John[4], Y. A. Garde[5] and R. R. Waghunde[6]

[1]Dept. of Plant Pathology, NMCA, NAU, Navsari, Gujarat (396 450), India
[2]Dept. of Plant Pathology, MRRC, SWMRU, NAU, Navsari, Gujarat (396 450), India
[3]Dept. of Entomology, NMCA, NAU, Navsari, Gujarat (396 450), India
[4]Dept. of Plant Pathology, [5]Dept. of Agricultural Statistics, NMCA, NAU, Navsari, Gujarat (396 450), India
[6]Dept. of Plant Pathology, College of Agriculture, NAU, Bharuch, Gujarat (392 012), India

Corresponding Author

Bhukya Srinivas
e-mail: bhukyasrinivas954@gmail.com

Article History

Received on 24th December, 2023
Received in revised form on 28th January, 2024
Accepted in final form on 20th February, 2024

Abstract

The present study was conducted during *kharif* (July-November, 2020) at the Department of plant pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat (396 450), India to examine the efficacy of four recognized antagonists and a control against *Xanthomonas oryzae* pv. *oryzae*. By employing paper disc method the results indicated that *Bacillus subtilis* exhibited the highest radius of inhibition. Following this, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Trichoderma viride* were identified as the next most effective antagonists. In addition, ten different botanical extracts, including a control, were examined for their inhibitory impact on the bacterial growth of bacterial blight at a 5% concentration using the poisoned food technique. Among these extracts, garlic clove extract demonstrated the maximum inhibition radius against the pathogen, followed by tulsi leaf extract and neem leaf extract, attributed to potent toxic principles. Conversely, extracts from karanj, nilgiri, dhatura, jetropha and rhizomes of ginger, turmeric and onion bulb were found to be less effective against *Xanthomonas oryzae* pv. *oryzae*. The goal of this study provided valuable insights to determine the potential antagonists and botanicals against bacterial blight of rice in vitro with experimental design of Completely Randomized Design which offer a cost effective solution for the disease, in increasing crop yield to the rice growing farmers of Gujarat without disturbing ecosystem and without creating the problems of pollution.

Keywords: Antagonists, botanicals, inhibition, *in vitro*, *Oryza sativa*, *Xoo*

1. Introduction

Rice (*Oryza sativa* L.) is a staple food crop that feeds over 70% of the world's population and is the most cost-effective source of protein and energy. India has the highest rice acreage with an annual production of 122.27 mt in 2020–21 (Indiastat, 2021). In Gujarat it is cultivated under area of 0.84 mha with production of 1.93 mt (Anonymous, 2019). One of the most widely cultivated food crops globally, yet its production is continually hampered by diseases induced by bacteria, viruses and fungi. Bacterial leaf blight (BLB) or bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Swings et al., 1990), remains a particularly detrimental rice disease in both rainfed and irrigated environments. The widespread occurrence of seed-borne *Xoo* has significant repercussions on rice production across diverse regions, including Asia, Australia, Latin America, Africa and the United States (Khush and Virmani (1985), Lozano (1977), Mew et al. (1993), Mew et al. (1987)). This bacterial pathogen contributes to substantial crop losses, reaching up to 50% Gnanamanickam et al. (1999). Compounding this issue is the presence of various races and pathotypes of the bacterium characterized by high variability, which poses formidable challenges in developing comprehensive and effective management strategies Mew et al. (1993). Explorations into the mechanisms of disease suppression by plant products have indicated that the active principles within these products may exert their effects directly on the pathogen, as highlighted by studies such as

Amadioha (2000), Ansari (1995). Conducting *invitro* testing of phytoextracts, biocontrol agents, fungicides and antibiotics provides essential preliminary insights into their efficacy against diseases within a relatively short time frame Jonit et al. (2016). Previous studies, such as Jambhulkar et al. (2018), explored the biocontrol potential of *Trichoderma harzianum* strain Th3 and *Pseudomonas fluorescens* strain RRb11, along with the fungicide carbendazim, against *Xoo*. While *P. fluorescens* RRb11 alone effectively controlled *Xoo*, the combination with *T. harzianum* Th3 and carbendazim proved inefficacious. Abdallah et al. (2019) investigated the effects and mechanisms of *Paenibacillus polymyxa* Sx3 on growth promotion and bacterial blight suppression in rice. Naqvi et al. (2018) examined extracts from water from 15 distinct plant parts, either separately or in combination, using *in vitro* poison food and disk diffusion techniques. Seven effective extracts, including *Mentha piperita* L., *Azadirachta indica* A. Juss and *Aloe barbadensis* Miller, showed efficacy against *Xoo* in glasshouse and field experiments. Sonika et al. (2020) conducted screenings of *Xoo* cultures with plant extracts, such as neem leaf, garlic bulb, onion bulb, ginger rhizome and tulsi leaf at varying concentrations (10%, 20% and 30%). Notably, garlic exhibited the maximum inhibition zone (9.2 mm), followed by tulsi (9.14 mm) at a 30% concentration. Srinivasan et al. (1959) from Maharashtra state in India were the first to describe *Xoo*. It is a typical vascular disease with a systemic origin. At the seedling stage, the "Kresek" phase is the most damaging (Chahal, 2005). The biology of cereals is modelled after rice, a food that is consumed everywhere (Bennetzen and Ma, 2003, Ronald and Leung, 2002, Shimamoto and Kyozuka, 2002). In Gujarat, major rice growing area is confined in the districts of Navsari, Valsad, Surat, Dangs, Panchmahal, Vadodara, Kheda and Ahmedabad (Verma and Shukla, 2011). Survey reports from experts at N.A.U., Navsari, consistently document the occurrence of an annual bacterial blight outbreak in South Gujarat (Anonymous, 2018).

2. Materials And Methods

The study was conducted during *kharif* (July–November, 2020) at the Department of plant pathology, Navsari Agricultural University, Navsari, Gujarat, India. The antagonistic properties of four known antagonists against *Xoo* (Table 1).

2.1. Paper disc method

The fungal antagonists were cultured in sterilized potato dextrose broth, while the bacterial antagonists were grown in sterilized nutrient broth, using 50 ml of medium in 150 ml conical flasks. Incubation periods were set at 10 days for fungal antagonists and 96 hours for bacterial antagonists within a B.O.D. incubator, maintaining a temperature of (27±2) °C.

For agar plate preparation, Petri plates were filled with sterilized nutrient agar medium and allowed to solidify. A bacterial suspension of *Xoo* at a concentration of 10^9 cells ml^{-1} (0.1 ml) was pipetted onto the center of the solidified plates and evenly spread using a sterilized spreader. Filter paper discs with a diameter of 5 mm were immersed in a previously prepared suspension (10^8 cells or spores ml^{-1}) of antagonists. Four such discs were strategically positioned equidistantly, 2 cm away from the center, on the inoculated plates. Each experimental setup was replicated three times. Control plates were established by placing discs dipped in sterilized distilled water. All labeled plates were then incubated at (27±2)°C in a B.O.D. for subsequent observation and analysis. Zone of inhibition radius were recorded at 24, 48 and 96 hour of incubation.

Table 1: List of different treatments of bioagents

a)	Treatments	5 (Five) T_1: *Trichoderma viride* (Navsari isolate) T_2: *Trichoderma harzianum* (Navsari isolate) T_3: *Pseudomonas fluorescens* (Navsari isolate) T_4: *Bacillus subtilis* (Navsari isolate) T_5: Control
b)	Experimental Design	CRD (Completely Randomized Design)
c)	Repetitions	5 (Five)
d)	Method	Paper disc method

2.1. Details of experiment

Table 2: Effect of Botanicals against the Pathogen *in vitro*

a)	Treatments	11 (Eleven)
b)	Experimental design	CRD (Completely randomized design)
c)	Repetitions	3 (Three)
d)	Method	Poisoned food technique

2.2. Poisoned food technique

The utilization of healthy and fresh plant parts, including leaves, bulbs, rhizomes and cloves, formed the basis of this study. These plant parts underwent a thorough cleaning process, including washing with fresh water and a final rinse with sterilized distilled water. Subsequently, 50 g of the plant parts were finely cut and minced with a grinder, incorporating 50 ml of sterilized distilled water. The resulting phytoextracts were filtered through a double-layered sterile muslin cloth into a 150 ml conical flask, sealed with non-absorbent cotton. The filtered extracts were then autoclaved at a pressure of 1.2 kg cm^{-2} for 20 minutes, establishing them as 1:1 extracts. The experimental plates were filled with sterilized nutrient agar medium and allowed to solidify. A bacterial suspension 0.1 ml was placed in the center of the solidified plates and evenly spread across the surface using a sterile spreader. Sterile filter paper discs, each with a diameter of 5 mm, were aseptically dipped into the 1:1 extract of each plant species. Four such discs were strategically positioned 2 cm away from the

center, maintaining equal distances. Three replications were maintained for each treatment, while plates with discs dipped in sterile distilled water served as controls. All inoculated plates were appropriately labeled and then incubated at room temperature (27±2)°C for further observation and analysis. Zone of inhibition radius were recorded at 24, 48 and 96 hour of incubation (Table 3).

Table 3: List of different phytoextracts tested *in vitro* against *Xoo*

Tr. No.	Common Name	Botanical name	Plant parts for extracts	Conc. (%)
T_1	Onion	*Allium cepa* L.	Bulb	5%
T_2	Neem	*Azadirachta indica* L.	Leaves	5%
T_3	Tulsi	*Ocimum sanctum* L.	Leaves	5%
T_4	Garlic	*Allium sativum* L.	Cloves	5%
T_5	Ginger	*Zingiber officinalis* Rosa	Rhizome	5%
T_6	Nilgiri	*Eucalyptus citridora* Hook	Leaves	5%
T_7	Karanj	*Pongamia glubra* L.	Leaves	5%
T_8	Dhatura	*Datura stamoneum* L.	Leaves	5%
T_9	Jetropha	*Jetropha curcas* L.	Leaves	5%
T_{10}	Turmeric	*Curcuma longa* L.	Rhizome	5%
T_{11}	Control	-	-	-

3. Results and Discussion

This experiment assessed the antagonistic activity of four distinct bioagents against *Xoo* in an *in vitro*. The results demonstrated that all screened antagonists exhibited greater efficacy compared to the control. Table 4, indicated that among the various biocontrol agents tested against *Xoo*, *Bacillus subtilis* displayed the highest zone of inhibition radius (4.97 mm) across all three observation periods. Following this, *Pseudomonas fluorescens* exhibited the next best performance with a zone of inhibition radius of (4.66 mm), followed by *Trichoderma harzianum* (2.06 mm) and *T. viride* (1.56 mm). The control treatment, in contrast, failed *Xoo*. The experiment distinctly revealed the effectiveness of local strains of bacterial bioagents (Navsari isolates) of *B. subtilis* and *P. fluorescens*, as evaluated against *Xoo*. Additionally, fungal bioagents, namely *T. harzianum* and *T. viride*, consistently demonstrated antagonistic activity against *Xoo*.

Table 4: Effect of antagonists against the pathogen *in vitro*

Tr. No.	Antagonists	Inhibition radius (mm)			
		24 hrs.	48 hrs.	96 hrs.	Mean
T_1	*Trichoderma viride*	1.79 (1.73)	1.76 (1.62)	1.68 (1.35)	1.74 (1.56)
T_2	*Trichoderma harzianum*	1.93 (2.23)	1.88 (2.07)	1.84 (1.90)	1.88 (2.06)
T_3	*Pseudomonas fluorescens*	2.52 (4.88)	2.47 (4.63)	2.44 (4.47)	2.48 (4.66)
T_4	*Bacillus subtilis*	2.57 (5.15)	2.54 (4.96)	2.51 (4.81)	2.54 (4.97)
T_5	Control (Untreated)	1.22 (0.00)	1.22 (0.00)	1.22 (0.00)	1.22 (0.00)
SEm±		0.001	0.002	0.009	0.002
CD (*p*=0.01)		0.004	0.005	0.026	0.005
C.V (%)		0.154	0.171	0.999	0.172

Figures in parentheses are original values; Figures outside parentheses are square root +0.5 transformed values

The findings of this experiment align with previous research, Manmeet and Thind (2002) evaluated *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma harzianum* and *Penicillium notatum* against *Xoo* and observed inhibitory

Table 5: Inhibition radius due to various phytoextract

Tr. No.	Phytoextract	Inhibition radius (mm)			
		24 hrs.	48 hrs.	96 hrs.	Mean
T_1	Onion	2.08 (2.83)	1.98 (2.42)	1.91 (2.17)	1.99 (2.47)
T_2	Neem	2.43 (4.40)	2.23 (3.48)	2.11 (2.98)	2.26 (3.62)
T_3	Tulsi	2.47 (4.62)	2.31 (3.87)	2.22 (3.45)	2.34 (3.98)
T_4	Garlic	2.85 (6.67)	2.77 (6.17)	2.72 (5.92)	2.78 (6.25)
T_5	Ginger	1.71 (1.43)	1.56 (0.93)	1.39 (0.43)	1.56 (0.93)
T_6	Nilgiri	1.75 (1.58)	1.60 (1.08)	1.44 (0.58)	1.60 (1.08)
T_7	Karanj	1.74 (1.53)	1.59 (1.03)	1.51 (0.78)	1.61 (1.11)
T_8	Dhatura	2.21 (3.38)	2.09 (2.88)	2.01 (2.55)	2.10 (2.93)
T_9	Jetropha	1.89 (2.08)	1.75 (1.58)	1.68 (1.33)	1.77 (1.66)
T_{10}	Turmeric	1.81 (1.78)	1.66 (1.28)	1.59 (1.03)	1.69 (1.36)
T_{11}	Control	1.22 (0.00)	1.22 (0.00)	1.22 (0.00)	1.22 (0.00)
SEm±		0.010	0.010	0.011	0.010
CD (*p*=0.01)		0.028	0.030	0.033	0.030
C.V (%)		0.823	0.931	1.058	0.933

Figures in parentheses are original values; Figures outside parentheses are square root +0.5 transformed values

effects by *B. subtilis, P. fluorescens* and *T. harzianum*. Additionally, Kaur and Thind (2002) studied *Pseudomonas fluorescens* through dual culture and reported inhibition zones of 4.24 mm against *Xoo*.

In this experiment, ten botanical extracts from various plant families were examined for their inhibitory effects on *Xoo* under *in vitro* conditions at a 5% concentration. The results, presented in Table 5, revealed that garlic extract exhibited the maximum zone of inhibition radius (6.25 mm), followed by tulsi leaf extract (3.98 mm), neem leaf extract (3.62 mm), dhatura leaf extract (2.93 mm), onion bulb extract (2.47 mm), jetropha leaf extract (1.66 mm), turmeric rhizome extract (1.36 mm), karanj leaf extract (1.11 mm), nilgiri leaf extract (1.08 mm) and ginger rhizome extract (0.93 mm) during 24, 48, and 96 hours of observations. The presence of toxic compounds like allicin, eugenol and azadirachtin in garlic, tulsi and neem extracts, respectively, indicates their potential to directly impact the growth of *Xoo*, the causal agent of bacterial blight in rice. These results corroborate those of Sonika et al. (2020), who reported maximum inhibition zones in garlic (9.2 mm) and tulsi (9.14 mm). Kumar (2006) who reported inhibition by Nilgiri, Lantana, Kali basuti and Ram ban. The inhibitory effect of these phytoextracts is first time reported against *Xoo*, whereas, Rajeswari (1991) reported that the antibacterial activity of Gandobaval against bacterial pathogen might be explain to have high level of glycoprotein and tannin. Additionally, Solanky (1983) found that extracts from garlic bulb, datura-white and bhoi-ringni exhibited bactericidal properties, inhibiting *Xoo*.

4. Conclusion

The investigation assessed four known antagonists against *Xoo* through the paper disc method, revealing *Bacillus subtilis* as the most effective with the highest inhibition radius. Meanwhile, utilizing the poisoned food technique, 10 plant extracts at 5% concentration were studied. Notably, garlic clove extract displayed the greatest inhibition radius, followed by tulsi and neem leaf extracts, showcasing their efficacy due to potent toxic principles. Conversely, karanj, nilgiri, dhatura, jetropha, ginger, turmeric and onion extracts exhibited comparatively lower effectiveness against *Xoo*.

5. Acknowledgement

We are grateful to the Dean of Post Graduate Studies, Navsari Agricultural University, Navsari for providing the necessary facilities to conduct the research. The author is also thankful to the Professor and Head, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari for providing facilities throughout the research work.

6. References

Abdallah, Y., Yang, M., Zhang, M., Masum, M.M., Ogunyemi, S.O., Hossain, A., Li, B., 2019. Plant growth promotion and suppression of bacterial leaf blight in rice by *Paenibacillus polymyxa* Sx3. Letters in Applied Microbiology 68(5), 423–429.

Amadioha, A.C., 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Protection 19(5), 287–290.

Anonymous, 2021. Indiastat. 2021. Available at https://www.indiastat.com/data/agriculture/rice/data-year/2021. Accessed on 25.12.2021.

Anonymous, 2018. Annual Report, Main Rice Research Centre, Plant Pathology., NAU, Navsari, 2–5.

Anonymous, 2019. First advance estimates of production of food grains for 2019-20. Ministry of agriculture and farmer welfare, 1. Source: http;//agricoop.gov.in

Ansari, M.M., 1995. Control of sheath blight of rice by plant extracts. Indian Phytopathology 48(3), 268–270.

Bennetzen, J.L., Ma, J., 2003. The genetic colinearity of rice and other cereals on the basis of genomic sequence analysis. Current Opinion Plant Biology 6(1), 128–133.

Chahal, S.S., 2005. Disease scenario in rice during post green revolution era in Punjab. Plant Diseases Research 20(1), 2–3.

Gnanamanickam, S.S., Priyadarisini, V.B., Narayanan, N.N., Vasudevan, P., Kavitha, S., 1999. An overview of bacterial blight disease of rice and strategies for its management. Current Science, 1435–1444.

Jambhulkar, P.P., Sharma, P., Manokaran, R., Lakshman, D.K., Rokadia, P., Jambhulkar, N., 2018. Assessing synergism of combined applications of *Trichoderma harzianum* and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice. European Journal of Plant Pathology 152(3), 747–757.

Jonit, N.Q., Low, Y.C., Tan, G.H., 2016. *Xanthomonas oryzae* pv. *oryzae*, biochemical tests, rice (*Oryza sativa*), bacterial leaf blight (BLB) disease, sekinchan. Journal of Applied and Environmental Microbiology 4(3), 63–69.

Kaur, M., Thind, B.S., 2002. Development of formulation of *Pseudomonas fluorescens* for control of Bacterial Blight of rice. Journal of Mycology Plant Pathology 32(3), 406–407.

Khush, G.S., Virmani, S.S., 1985. Breeding rice for disease resistance. Progress in Plant Breeding 1, 239–279.

Kumar, A., 2006. Evaluation of botanicals against major pathogens of rice. Indian Phytopathology 59(4), 509–511.

Lozano, J.C., 1977. Identification of bacterial leaf blight in rice, caused by *Xanthomonas oryzae*, in America. Plant Disease Reporter 61(8), 644–648.

Manmeet, M., Thind, B.S., 2002. Management of bacterial blight of rice with bioagents. Plant Diseases Research 17(1), 21–28.

Mew, T.W., 1987. Current status and future prospects of research on bacterial blight of rice. Annual Review of Phytopathology 25(1), 359–382.

Mew, T.W., Alvarez, A.M., Leach, J.E., Swings, J., 1993. Focus

on bacterial blight of rice. Plant Disease 77(1), 5–12.
Naqvi, S.A.H., Umar, U.D., Hasnain, A., Rehman, A., Perveen, R., 2018. Effect of botanical extracts: A potential biocontrol agent for *Xanthomonas oryzae* pv. *oryzae*, causing bacterial blight disease of rice. Pakistan Journal of Agriculture Sciences 32(1), 59–72.
Rajeswari, E., 1991. Effect of plant derivaties on rice blast pathogen (*Pyricularia oryzae*). M.Sc. (Agri.) Thesis TNAU, Coimbatore, India, 129.
Ronald, P., Leung, H., 2002. The rice genome: The most precious things are not jade pearls. Science 296, 58–59.
Shimamoto, K., Kyozuka, J., 2002. Rice as a model for comparative genomics of plants. Annual Review of Plant Biology 53, 399–419.
Solanky, K.U., 1983. Problems associated with the control of bacterial blight (*Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye) of rice. Thesis M.Sc. (Agri.) Gujarat Agricultural University, S.K. Nagar, India, 65.
Sonika, D., Durga, P., Subhashish, S., 2020. Evaluation of antibiotics, fungi toxicants and botanicals against *Xanthomonas oryzae* pv. *oryzae*, a cause of bacterial blight of rice. International Journal of Plant Protection 13(1), 1–8.
Srinivasan, M.C., Thirmulachar, M.J., Patel, M.K., 1959. Bacterial blight of rice. Current Science 28(1), 469–470.
Swings, J., Van den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T.W., Kersters, K., 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as Pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. International Journal of Systematic and Evolutionary Microbiology 40(3), 309–311.
Verma, D.K., Shukla, K., 2011. Nutritional value of rice and their importance. Indian Farmers Digest 44(1), 21–35.

