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# Management of bacterial leaf blight of rice using plant extracts

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**Abstract**
*Xanthomonas oryzae* pv. *oryzae*, the incitant of bacterial leaf blight disease of rice acts as a bottleneck in productivity of rice. Chemical control measures impose hazards to human as well as soil health. So, there is always a quest for eco-friendly management strategies. *In vitro* and *in vivo* assays were employed to evaluate the sensitivity of the pathogen against crude aqueous extracts of different plants. Out of five plant extracts, *Datura metel* was most effective in restricting the pathogen followed by *Allium sativum*. The percent inhibition exhibited by *D. metel* extract were 46.73% and 58.04% at 15% and 20% concentrations, respectively. Aqueous extract spray of *D. metel* at 20% proved effective in restricting the disease by 11.9% and recorded 104% yield increase over control.

**Keywords:** Bacterial leaf blight, Rice, plant extracts

## Introduction
Rice (*Oryza sativa* L.) is an essential source of carbohydrate for major portion of the global population. In India, there has been a slump of area under production from last 20 years. Odisha ranks third and fourth among other states in terms of area and production respectively, during 2016-17 (http://eands.dacnet.nic.in). Rice, being an essential component of human diet, there is a high demand to increase its productivity. But a number of biotic as well as abiotic stresses cause hindrance in fulfilling the demand. Among the biotic stresses, disease-causing pests cause a significant yield loss. These pests include fungi, bacteria, virus, phytoplasma and nematodes. The major pests include *Pyricularia oryzae* (blast), *Bipolaris oryzae* (brown spot), *Rhizoctonia solani* (sheath blight) and *Xanthomonas oryzae* pv. *Oryzae* (bacterial leaf blight, BLB). This bacterium is Gram negative and belongs to family Xanthomonadaceae under Gammaproteobacteria (Nino, 2006) [10]. It enters the plant through the hydathodes and colonize the vascular tissues. At early stages of the crop, this disease appears as *Kresek* symptom whereas at tillering, it occurs with leaf blight symptom. The yield loss attributed to this disease is about 70% (Reddy *et al.,* 1979) [11]. Several management strategies employed to combat the disease include genetic resistance, use of chemicals, bioagents, etc. It is obvious that the use of bactericides helps in reducing the disease spread. But simultaneously they pose threat to environment and human health with the residual toxicity. An investigation was conducted at ICAR-NRRI, Cuttack using some plant extracts, under *in vitro* and *in vivo* conditions to find out the most effective extract for management of BLB.

## Materials and Methods
### Collection, Isolation, Purification and Pathogenicity Test
Fifty-two BLB disease samples were collected across the states of eastern India. In the laboratory, the leaves were cut into 0.5 to 1 cm bits. These leaf bits were stimulated to ooze in moist chamber for three to four hours (Kotasthane, 2003) [6]. Isolation of the pathogen was done aseptically using modified Wakimoto's agar (MWA) plate. For routine works, the isolate was maintained on MWA slants at 4 □. Glycerol stocks (25%) were prepared and stored at -80□ for long term preservation. Pathogenicity assays were conducted on TN1 cultivar (susceptible to BLB) using three days-old culture by leaf clipping (Kauffman *et al.,* 1973) [5]. All the isolates were subjected to pathotyping and the most virulent isolate was chosen for this study.

**Preparation of Plant Extracts and *in vitro* Antimicrobial Assay**

A preliminary screening of a number of plants was performed to evaluate their antibacterial efficacy using agar disc diffusion method (Baeur *et al.,* 1966) [1]. The plant parts were washed thoroughly with tap water and air dried for an hour. Required quantity of each part was weighed and ground with sterile water using a mortar and pestle. After volume makeup, they were filtered through a muslin cloth. A stock solution of 50% strength was prepared and diluted to 15% and 20% respectively. The experiment was conducted with six treatments, including control with four replications. Sterile discs (HiMedia Laboratories) of 6mm diameter were saturated with the plant extracts. Few loops of three days-old bacterial culture were homogenized in two millilitres autoclaved distilled water. A layer of bacterial suspension was spread over the solidified MWA plates and left for 30 minutes to air dry under laminar hood. At the centre of each inoculated plate, discs having extracts were placed and incubated at 27□. After 72 hours of incubation, the zone of inhibition (excluding the diameter of the disc) was recorded and percent inhibition was calculated as follows:

$$\% \ inhibition = \frac{Diameter\ of\ the\ zone\ of\ inhibition\ \times 100}{Diameter\ of\ the\ petriplate}$$

***In vivo* Antimicrobial Assay**

Required quantity of plant parts were collected and their aqueous extracts were prepared as mentioned previously. A total of 10 ten different treatments and a control was setup with three replications each. Rice cultivar TN1 was used for the pot experiment. The seedlings were transplanted at 21 days after sowing. Forty-five days old plants were clip-inoculated with the pathogen. A foliar spray of extracts was given 10 days after inoculation. Distilled water was sprayed on control. After 21 days of inoculation, the disease score (IRRI, 1996) was recorded. Percent disease index (PDI) was calculated as follows:

$$PDI\ (\%) = \frac{Sum\ of\ all\ blight\ scores \times 100}{Number\ of\ leaves\ scored \times Maximum\ rating}$$

The data on yield (g/pot) was recorded for all the replications and the average was calculated.

**Results and Discussion**

**Antimicrobial Assay under *in vitro* Conditions**

From Table-1, it was evident that the aqueous extracts showed an increased level of inhibitory activity with rise in concentration. At 15% strength, *Datura metel* recorded the highest zone of inhibition (3.9 cm), followed by *Allium sativum* extract (2.1 cm). Ajoene, an antimicrobial compound in garlic was reported to inhibit *Xanthomonas* species (Naganawa *et al.,* 1996) [9]. The antimicrobial property in *D. metel* is due to a biologically active compound, withametelin B (Meena *et al.,* 2013) [8] *Curcuma domestica* was the least effective of all the tested plant extracts. The extracts of *Achyranthes aspera* and *Justicia adhatoda* were at par in their effectivity. The extract of *J. adhatoda* contributed to checking the growth of *Xoo* (Govindappa *et al.,* 2011) [2]. The antimicrobial effect of *A. aspera* against *X. axonopodis* had been evaluated by previous studies (Manonmani *et al.,* 2009) [7]. Moreover, at 20% strength, the highest inhibitory extract was that of *D. metel* (4.9 cm), followed by *A. sativum* and *J. adhatoda*. Two plant extracts, *C. domestica* and *A. aspera* showed similar magnitude of inhibition of the pathogen.

**Antimicrobial Assay under *in vivo* Conditions**

At 20% concentration, *D. metel* spray was the most effective in disease suppression followed by *A. sativum* extract. *A. aspera, J. adhatoda* and *C. domestica* were at par with control on the basis of disease severity. The disease reduction varied between 0 and 12%. Highest yield was achieved by using *Datura* aqueous extract (24.1 g/pot) followed by garlic extract (22 g/pot) at 20% strength. At both the strength, foliar spray with *C. domestica* provided the lowest yield increase over control (10-13%) (Table-2). Turmeric and *D. metel* extracts checked the growth of *Xoo* under *in vitro* condition as well minimized the disease spread in rice plant (Yugander *et al.,* 2015) [12].

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**Table 1:** *In vitro* evaluation of bioefficacy of plant extracts against *Xanthomonas oryzae* pv. *Oryzae*

| Treatment | Plant extracts | Parts used | Concentration (%) | | | |
|---|---|---|---|---|---|---|
| | | | 15 | | 20 | |
| | | | Mean zone of inhibition (cm) | % inhibition | Mean zone of inhibition (cm) | % inhibition |
| T1 | Garlic (*Allium sativum*) | Cloves | 2.88 (1.84)* | 34.23 | 4.18 (2.16) | 49.70 |
| T2 | Chaff-flower (*Achyranthes aspera*) | Leaves | 2..05 (1.60) | 24.41 | 2.48 (1.73) | 29.46 |
| T3 | Adhatoda (*Justicia adhatoda*) | Leaves | 2.20 (1.64) | 26.19 | 2.95 (1.86) | 35.12 |
| T4 | Turmeric (*Curcuma domestica*) | Rhizome | 1.48 (1.41) | 17.56 | 2.48 (1.73) | 29.46 |
| T5 | Indian Thornapple (*Datura metel*) | Leaves | 3.93 (2.10) | 46.73 | 4.88 (2.32) | 58.04 |
| T6 | Control | | 0.00 (0.71) | | 0.00 (0.71) | |
| | SE(m)± | | 0.01 | | 0.01 | |
| | C.D. | | 0.03 | | 0.02 | |

* Data in parenthesis indicate √ (x + 0.5) transformed values

**Table 2:** *In vivo* evaluation of bioefficacy of plant extracts against *Xanthomonas oryzae* pv. *Oryzae*

| Treatment | Plant extracts with concentration | Mean PDI (%) | % reduction over control | Mean yield (g/pot) | % increase over control |
|---|---|---|---|---|---|
| T1 | Garlic (*Allium sativum*) @15% | 100.0(90.0)* | 0.0 | 19.1 | 61.8 |
| T2 | Garlic (*Allium sativum*) @20% | 91.9 (76.5) | 8.1 | 22.0 | 86.8 |
| T3 | Chaff-flower (*Achyranthes aspera*) @15% | 100.0 (90.0) | 0.0 | 15.5 | 31.2 |
| T4 | Chaff-flower (*Achyranthes aspera*) @20% | 100.0 (90.0) | 0.0 | 18.1 | 53.3 |

| | | | | | |
|---|---|---|---|---|---|
| T5 | Adhatoda (*Justicia adhatoda*) @15% | 100.0 (90.0) | 0.0 | 18.5 | 56.6 |
| T6 | Adhatoda (*Justicia adhatoda*) @20% | 100.0 (90.0) | 0.0 | 18.5 | 57.1 |
| T7 | Turmeric (*Curcuma domestica*) @15% | 100.0 (90.0) | 0.0 | 13.0 | 10.2 |
| T8 | Turmeric (*Curcuma domestica*) @20% | 100.0 (90.0) | 0.0 | 13.4 | 13.4 |
| T9 | Indian Thornapple (*Datura metel*) @15% | 97.0 (84.2) | 3.0 | 19.6 | 66.2 |
| T10 | Indian Thornapple *(Datura metel*) @20% | 88.1 (70.1) | 11.9 | 24.1 | 104.0 |
| T11 | Control | 100.0 (90.0) | | 11.8 | |
| | SE(m)± | 2.84 | | 0.32 | |
| | C.D. | 8.37 | | 0.94 | |

*Data in parenthesis indicate arcsine transformed values

## References


1. Bauer AW, Kirby WMM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. American Journal of Clinical Pathology. 1966; 45:493-496.
2. Govindappa M, Umesha S, Lokesh S. *Adhatoda vasica* leaf extract induces resistance in rice against bacterial leaf blight disease (*Xanthomonas oryzae* pv. *oryzae*). International Journal of Plant Physiology and Biochemistry. 2011; 3(1):6-14.
3. http://eands.dacnet.nic.in. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India.
4. IRRI. SES (Standard Evaluation System for Rice). International Network for Genetic Evaluation of Rice. Los Baños: International Rice Research Institute (IRRI), 1996.
5. Kauffman HE, Reddy APK, Hsieh SPV, Marca SD. An improved technique for evaluation of resistance of rice varieties to *Xanthomonas oryzae*. Plant Disease Reporter. 1973; 57:537-541.
6. Kotasthane AS. A simple technique for isolation of *Xanthomonas oryzae pv oryzae*. Journal of Mycology and Plant Pathology. 2003; 33:277-278.
7. Manonmani K, Jayasekhar M, Gailce Leo Justin C, Trangaselvabai T. Identification of active principle in the herbal extracts possessing bactericidal in the herbal extracts possessing bactericidal action against citrus canker, *Xanthomonas axonopodis* pv. *citri*. Indian Journal of Agricultural Research. 2009; 43(2):129-133.
8. Meena C, Gopalakrishnan J, Dureja P. Antibacterial Withametelin B from *Datura metel* against *Xanthomonas oryzae* pv. *Oryzae*. Biopesticides International. 2013; 9(1):31-37.
9. Naganawa R, Iwata N, Ishikawa K, Fukuda H, Fujino T, Suzuki A. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. Applied and Environmental Microbiology. 1996; 62:4238-4242.
10. Nino-Liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Molecular Plant Pathology. 2006; 7(5):303-324.
11. Reddy APK, Mackenzie DR, Rouse DI, Rao AV. Relationship of bacterial leaf blight severity to grain yield of rice. Phytopathology. 1979; 69:967-969.
12. Yugander A, Ladhalakshmi D, Prasad MS, Laha GS. Evaluation of selected plant extracts against *Xanthomonas oryzae* pv. *Oryzae* causing bacterial blight of rice. World Journal of Pharmaceutical Research. 2015; 4(10):1555-1561.