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# EVALUATION OF SELECTED PLANT EXTRACTS AGAINST *XANTHOMONAS ORYZAE* PV. *ORYZAE* CAUSING BACTERIAL BLIGHT OF RICE

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## ABSTRACT

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), remains a major production constraint in rice cultivation. The extent of yield loss could be as high as 50% and depends on the growth stages of the crop, cultivar, season, climatic condition and level of nitrogen fertilizer applied. Chemical control of the disease is not very successful. Use of botanicals in the management of plant diseases is gaining importance as it is safe and eco-friendly. In the present study, the efficacy of 33 plants extracts from different family was tested for their antibacterial activity against *Xoo*. Antimicrobial activity of aqueous plant extract was determined by agar well method on MWA agar medium. Among these extracts, six plant extracts exhibited significant antibacterial activity against *Xoo*. Out of these five plant extracts were evaluated under field condition for their effectiveness to reduce the BB. Out of these, three plants extract viz., *Curcuma domestica*, *Datura metel* and *Oscimum sanctum* were most effective which is reflected in reduced lesion length. All the treatments significantly increased the yield.

**KEYWORDS:** Bacterial blight of rice, *Xanthomonas oryzae* pv. *oryzae*, Pathogenicity.

## INTRODUCTION

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* (*Xoo*) is one of the most important diseases of rice and is a serious production constraint especially in the irrigated and rainfed lowland ecosystems. Major epidemics were reported in north western India and Kerala (Laha *et al.*, 2009). The disease appeared in epidemic form in

about 10,000 acres of rice variety, Samba Mahsuri in Kurnool district of Andhra Pradesh during 2010. Recently, the disease appeared in epidemic proportion in many parts of Andhra Pradesh during 2013 and 2014 (Yugander *et al.*, 2013; 2014). The disease has the potential to reduce the rice yield by 50% or more depending on the rice variety, stage of the plants and the climatic conditions. Chemical control of the disease is not very successful and none of the chemicals including antibiotics were found to provide sufficient level of protection under Indian conditions (Laha *et al*., 2009). Use of plant based botanical pesticides offer an alternative for the management of plant diseases. There are several reports which show antibacterial and antifungal nature of several plant extracts. Few studies have reported the efficacy of selected plant extracts of against bacterial blight of rice (Kagale *et al*., 2004; Meena *et al*., 2013; Kumar *et al*., 2009; Gurjar *et al*., 2012), rhizome extract of *Acorus calamus* against brown spot of rice (Devi and Chettry, 2013) and leaf extracts of *Prosophis juliflora* and *Zizyphus jujube* against rice blast (Kamalakannan *et al*., 2001). In the present study, we tested the antibacterial activity of 33 plant extracts against *Xoo* and efficacy of selected plant extracts in reducing the bacterial blight severity under field condition.

## MATERIALS AND METHODS

**Isolation and maintenance of *Xoo*:** The pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was isolated from infected rice leaf sample collected from experimental fields of Indian Institute of Rice Research following standard isolation procedure (Yugander *et al*., 2014). The pure culture of *Xoo* was maintained in modified Wakimoto's slants at $4^0$C for short term storage and in 15% glycerol at $-80^0$C for long term storage. The pathogenicity of the isolate was confirmed by inoculating on TN1 plants.

**Preparation of Plant Extracts:** Different plant parts (leaf, root, stem, flower buds, rhizome, bark, seeds, bulbs and fruits) of 33 different plant species belonging to different family were collected from fields in and around Indian Institute of Rice Research, Rajendranagar, Hyderabad or commercially (Table 1). The plant parts were thoroughly washed 2-3 times in running tap water followed by sterilized distilled water. The crude extracts of different plant parts were prepared in a sterile mortar and pestle separately using sterile distilled water (1:1 w/v) and filtered though muslin cloth. The filtrate was centrifuged at 15000 rpm for 10 min and the clear supernatant was collected. These extracts were then passed through bacteria proof sterile syringe filters (Axiva filters, SFPV13R). This pure extract was considered as

100% (concentrated) and was directly tested for its antimicrobial effect against *Xoo* under *in vitro* condition.

**Testing of plant extracts for antimicrobial activity:** Antimicrobial activity of different aqueous plant extracts was determined by agar well method on modified Wakimoto's agar (MWA) medium. Freshly prepared sterile MWA medium was uniformly poured in sterile plastic Petri plates (15 ml/plate). Once the medium was solidified, each plate was uniformly smeared with culture of *Xoo* by putting 100 µL bacterial suspensions (3 days old) and then spreading with a sterile spreader. In each plate, 4 wells (5 mm diameter) were made in with the help of sterile cork borer and in each well 50 µL individual plant extract was added. The plates were then incubated in undisturbed condition at $27^0$C for 72 h and the diameter of inhibition zones was measured.

**Field evaluation of selected plant extracts:** The effect of selected plant extracts on bacterial blight of rice was tested under field condition (cultivar TN1). The variety was raised in 3x2 $m^2$ plots following recommended package of practices. In each plot, one square meter area (block) was marked with wooden sticks when the plants were in maximum tillering stage. Three blocks (replications) were kept for each treatment. The selected plant extracts prepared in sterile distilled water (1:1) were sprayed in each marked blocks one day prior to bacterial inoculation (@ 330 ml/block). *Xoo* was grown in modified Wakimoto's medium at $27\pm1^0$C. Using 3-days old bacterial suspension (0.1 OD), plants were clip inoculated (Kauffman *et al*., 1973). Eight days after *Xoo* inoculation, another spray of respective plant extracts was given. Observations were taken on $8^{th}$ and $16^{th}$ day, after bacterial inoculation by measuring the lesion length. In each block, observations were taken on 10 individual leaves. Observations were also taken on any phytotoxic effects of these plant extracts. Plants from each one square meter area from each treatment and control treatments were harvested and yield per sq. mt was calculated.

## RESULTS

*Xoo* strain isolated and used in the present study produced typical susceptible reaction when inoculated on TN1 plants under glass house condition. Out of 33 plant extracts evaluated for their antibacterial activity, 6 plant species showed inhibitory effect against *Xoo* under *in vitro* condition (Table 1). These include leaf extracts of *Eucalyptus globulus*, Datura metel and *Ocimum sanctum*, modified stem extracts of *Allium sativum* and *Curcuma domestica* and extract of flower buds of *Syzygium aromaticum*. The mean diameter of inhibition zone varied

from 1.33 cm in case of *Eucalyptus globulus* to 3.73 cm in case of *Allium sativum* (Table 1). In case of *Eucalyptus globulus*, the bacterial growth around the inhibition zone turned blackish in colour. However, when such bacterial growth was cultured on fresh MWA medium, they showed normal yellow growth. Out of the six plant extracts which showed inhibitory effects against *Xoo*, five plant extracts were evaluated under field condition for their effectiveness to reduce the bacterial blight disease intensity. All the plant extracts significantly reduced the lesion length (Table 2). Of these five plant extracts, *Curcuma domestica*, *Datura metel* and *Oscimum sanctum* were most effective which is reflected in reduced lesion length on both dates of observation (Table 2). All the treatments also significantly increased the yield (Table 2). No phytotoxic symptoms were observed with any of the plant extracts.

**Table 1: Antibacterial effect of extracts of different plant species against *Xanthomonas oryzae* pv. *Oryzae*.**

| Plant Name | Common names | Family | Part Used | Mean inhibition zone (cm) ± SE |
|---|---|---|---|---|
| *Allium sativum* | Garlic | Amaryllidaceae | stem | 3.73±0.16 |
| *Curcuma domestica* | Turmeric | Zingiberaceae | Stem | 2.18 ±0.27 |
| *Syzygium aromaticum* | Clove | Myrtaceae | Flower buds | 1.48 ±0.22 |
| *Eucalyptus globulus* | Eucalyptus | Myrtaceae | Leaf | 1.33 ± 0.14 |
| *Datura metel* | Thorn apple | Solaneceae | Leaf | 1.8 ± 0.21 |
| *Ocimum sanctum* | Holy Basil | Lamiaceae | Leaf | 2.23 ± 0.07 |
| *Tamarindus indica* | Tamarind | Fabaceae | Leaf | 0 |
| *Mangifera indica* | Mango | Anacardiaceae | Leaf | 0 |
| *Azadirachta indica* | Neem | Meliaceae | Leaf | 0 |
| *Zingiber officinale* | Ginger | Zingiberaceae | Rhizome | 0 |
| *Cinnamomum camphorum* | Camphor | Lauraceae | Bark | 0 |
| *Aloe vera* | Indian Aloe | Liliaceae | Succulent | 0 |
| *Piper nigrum* | Black pepper | Piperaceae | Seeds | 0 |
| *Ricinus communis* | Castor | Euphorbiaceae | Leaf | 0 |
| *Allium cepa* | Onion | Amaryllidaceae | Bulb | 0 |
| *Lantana camara* | Red Sage | Verbanaceae | Leaf | 0 |
| *Tridax procumbens* | Coat button | Asteraceae | Leaf | 0 |
| *Tagetes patula* | Marigold | Asteraceae | Leaf | 0 |
| *Parthenium hysterophorus* | Congress Grass | Asteraceae | Leaf | 0 |
| *Catharanthus rosea* | Periwinkle | Apocynaceae | Leaf | 0 |
| *Calotropis gigantea* | Crown flower | Asclepidaceae | Leaf | 0 |
| *Ageratum conyzoides* | Goat weeds | Asteraceae | Leaf | 0 |
| *Manilkara zapota* | Sapota | Rutaceae | Leaf | 0 |
| *Ipomea batatas* | Sweet potato | Convolvulaceae | Leaf | 0 |
| *Hybiscus rosa sinensis* | China rose | Malvaceae | Leaf | 0 |
| *Carica papaya* | Papaya | Caricaceae | Leaf | 0 |

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|---|---|---|---|---|
| *Euphorbia hirta* | Lal Dudhi | Euphorbiacae | Leaf | 0 |
| *Zigiphus jujuba* | Red date | Rhamnaceae | Leaf | 0 |
| *Cassia auriculata* | Avaram | Fabaceae | Leaf | 0 |
| *Withania somnifera* | Ashwagandha | Solanaceae | Root | 0 |
| *Aegle marmelos* | Bael | Rutaceae | Leaf | 0 |
| *Pongamia pinnata* | Pongam | Fabaceae | Leaf | 0 |
| *Capsicum annuum* | Red pepper | Solanaceae | Fruit | 0 |

**Table 2: Effect of selected plant extracts on lesion length caused by *Xanthomonas oryzae* pv. *Oryzae.***

| Plant species | Mean Lesion Length (cm) | | Yield (gm/m$^2$) |
|---|---|---|---|
| | 8th day | 16th day | |
| *Allium sativum* | 2.5b | 9.4b | 513.3a |
| *Syzygium aromaticum* | 2.5b | 9.7b | 457.0b |
| *Datura metel* | 2.4b | 8.1c | 436.3bc |
| *Ocimum sanctum* | 2.3bc | 8.2c | 392d |
| *Curcuma domestica* | 2.1c | 7.9c | 406.7cd |
| Unsprayed Control | 4.9a | 14.1a | 315.3e |
| CV (%) | 8.46 | 5.13 | 4.48 |
| LSD (p=0.05) | 0.43 | 0.89 | 34.22 |

## DISCUSSION

There are reports which show antibacterial nature of extracts of *Datura metel*, *Curcuma longa* and *Eucalyptus grandifolia* against rice diseases (Kagale *et al*., 2004; Meena *et al*., 2013; Devi and Chettry, 2013). Meena *et al*. (2013) reported presence of the antimicrobial compound withametalin B in *Datura metel*. Maximum inhibition observed with *Allium sativum* may be due to the presence of sulphur compounds and allicin (Kurucheve and Padmavathi, 1998; Singh and Singh, 2005). Many research reports indicated possible use of plant extracts for management of plant diseases. Colpas *et al*. (2009) reported that aqueous extract of leaves of *Ocimum gratissimum* induced systemic resistance in cucumber against *Colletotrichum lagenarium* as reflected by reduction in disease incidence and an increase in chitinase production. Antibacterial nature of *Datura metal*, *Curcuma longa* and *Eucalyptus grandifolia* have been demonstrated in several studies (Kagale *et al*., 2004; Meena *et al*., 2013; Kumar *et al*., 2009). The antimicrobial properties of these plants has been attributed to the presence of chemical compounds *viz*., curcumin (in *Curcuma longa*), hyoscymine Scopolamine and withametalin B (in *Datura stramonium* and *D. metel*) and allicin (in *Allium sativum*) (Gurjar *et al*., 2012; Meena *et al*., 2013).

## CONCLUSIONS

The present study shows that some of these plant extracts can be used for the management of bacterial blight of rice under field condition. As the chemical control of this disease is not very successful, management of the disease with the locally available plant materials like Datura and Curcuma can be the best alternative.

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