

Detection of *Xanthomonas oryzae* pv *oryzae* in BLB paddy seed samples from endemic regions of Telangana state, India

Abstract

Bacterial Leaf Blight disease (BLB) in rice caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*), is one of the most economically important diseases causing epidemics globally, resulting in severe crop losses of 50% in addition to affecting the seed quality in Asia. In the present study, ten BLB affected paddy seed samples were collected during Kharif, 2021-22 from the farmer's fields of major BLB endemic districts viz., Nizamabad and Khammam of Telangana state. The collected BLB seed samples were subjected to different standard seed health tests (ISTA, 1996) for detection and isolation of *Xoo*. Among the different methods evaluated, the detection of *Xoo* from BLB affected seed samples was high in the Agar plate method followed by the test tube water agar method. However, the component plating method revealed that, of the different seed components, the per cent recovery of *Xoo* was high from the embryo followed by lemma, palea and endosperm indicating the systemic type of infection of *Xoo*. Further, the results of the paper towel method stated that of the ten BLB affected paddy seed samples collected, these seed samples of Govuru village (Nizamabad district) and Rejarla village (Khammam district) have recorded below Indian Minimum Seed Certification Standards i.e 77.00% and 79.25% of per cent seed germination compared to the seed samples of other locations.

Keywords: *Xoo*, systemic, seed rot, seed infection and pathogenicity

Introduction

Xanthomonas oryzae pv *oryzae* (*Xoo*), a bacterial pathogen causing bacterial leaf blight disease (BLB) in rice, can cause widespread disease and has caused epidemics globally, resulting in severe crop losses of 50% in Asia. The disease has become a serious problem in rice production in several rice growing states of India, especially in irrigated and rainfed lowland ecosystems (Mew, 1987). Among rice diseases, bacterial blight (BLB) is caused by *Xanthomonas oryzae* pv. *oryzae* has become a serious constraint for rice production. The farmers of the Fukuoka area, Japan first noticed the disease in 1884 (Tagami and Mizukami, 1962) and its bacterial nature was established and described by Ishiyama in 1922.

Healthy seed plays an important role in the successful cultivation of all types of crops. Crop response to other inputs largely depends on the quality seed. It is estimated that good quality seed alone can contribute about 18 to 20% increasing crop yield keeping all the other inputs constant. Seedborne pathogens are not merely the cause of the reduction of germination capability of crop seeds but are also responsible for the variation of plant morphology in the field and reducing yield up to 15 to 90%.

In Telangana state, it was known from regular disease monitoring surveys conducted in major paddy growing areas viz., Nizamabad, Warangal, Khammam and Nalgonda districts the occurrence of BLB every year in moderate to severe form where in some places of the state not only causing seedling mortality in a nursery but also affecting yield. Especially the disease has become endemic in the Nizamabad district. In the transplanted field, the disease causes, leaf blight symptoms which are characterized by wavy elongated lesions, along the leaf margins. However, in addition to the infection in grown up plants, the pathogen causes seedling mortality in the nursery resulting in poor seedling/population stand. Kresek is the result of seed infection that is observed in the seedling stage/ young plants and during the

tillering stage of susceptible cultivars. Therefore, a need was felt for the detection of *Xoo* from BLB seed samples to know the systematic nature of the bacterium in rice.

Materials and methods

During *Kharif*, 2021-22, Bacterial Leaf Blight (BLB) affected paddy seed samples were collected from major BLB endemic districts *viz.*, Nizamabad and Khammam of Telangana state at the time of harvesting in November. A total of 10 BLB affected paddy seed samples of BPT 5204 (Samba mashuri) with five seed samples from different locations representing each district were collected from farmer fields. The collected seed samples were kept at ambient storage conditions for further studies.

The collected BLB affected paddy seed samples were subjected to standard seed health tests (ISTA, 1996) to detect and isolate *Xoo*. For the isolation of the *Xoo* pathogen agar plate, test tube water agar, component plating and paper towel methods were used.

Agar plate method

Wakimoto and nutrient agar media were used to detect and isolate *Xoo*. Initially, the BLB affected paddy seed samples were surface sterilized with 1% sodium hypochloride solution for 45 sec and then washed twice with sterilized distilled water for 60 sec to remove excess chemical solution followed by air drying. Four seeds towards the periphery with one in the centre were placed at equidistance in the petri plates and incubated at $(28 \pm 2^{\circ}\text{C})$ for 3-4 days in the BOD incubator. After incubation, the bacterial colonies observed were recorded and expressed in percentage.

Test tube water agar method

Culture test tubes of 15 ml capacity were filled with 10 ml of 1 per cent water agar and allowed to solidify. After surface sterilization, one seed per test tube was placed and incubated at $28 \pm 2^{\circ}\text{C}$ in a BOD incubator. The seedlings were observed daily for symptom expression and the per cent seed infection by *Xoo* pathogen was recorded.

Paper towel method

The paper towels were initially soaked in sterile distilled water. After draining out excess water, one hundred seeds from each seed sample were randomly taken and placed on it at equidistance spacing on a moistened paper towel and covered with another moistened paper towel and rolled which were placed vertically in a walk-in type germinator, maintained at a constant temperature of $25 \pm 0.5^{\circ}\text{C}$ and $90 \pm 3\%$ R.H. Four replications for each seed sample were maintained.

Component plating method

Component plating was done according to the method described by Madenet *al.* (1975) to locate the *Xoo* in different components of the seed. Initially, the BLB affected paddy seeds were soaked for about 5 hrs in sterile water after which the seeds were separated into lemma, palea, embryo and endosperm using a surgical blade and forceps. These components were surface sterilized for about 30 seconds with 1 per cent NaOCl solution and each component was placed separately on WMA medium. The plates were then incubated at $28 \pm 2^{\circ}\text{C}$ for 3 days.

Percentages of seed germination, seed infection and seed rot are as follows

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

$$\text{Seed infection (\%)} = \frac{\text{Number of seeds infected by } Xoo}{\text{Total number of seeds}} \times 100$$

$$\text{Seed rot (\%)} = \frac{\text{Number of seeds decayed}}{\text{Total number of seeds}} \times 100$$

The isolated *Xoo* cultures were identified following cultural characters and staining and tested for pathogenicity. For proving the Koch postulates, Pure cultures of ten *Xoo* isolates grown separately on MWA medium at 28±2°C for 3-4 days was used. The culture plates were flooded with 10 ml of sterile distilled water and by scrapping with a sterile plastic inoculation loop, bacterial mass was gently removed and the bacterial suspension was adjusted to 10⁸-10⁹ cfu /ml. By following the leaf clipping method (Kauffman *et al.*, 1973), the suspension was used for artificial inoculation with help of scissors on BPT 5204 plants the highly susceptible cultivar at 28 days old seedlings (4 weeks). While the seedling leaves cut with scissors dipped in sterile distilled water served as control.

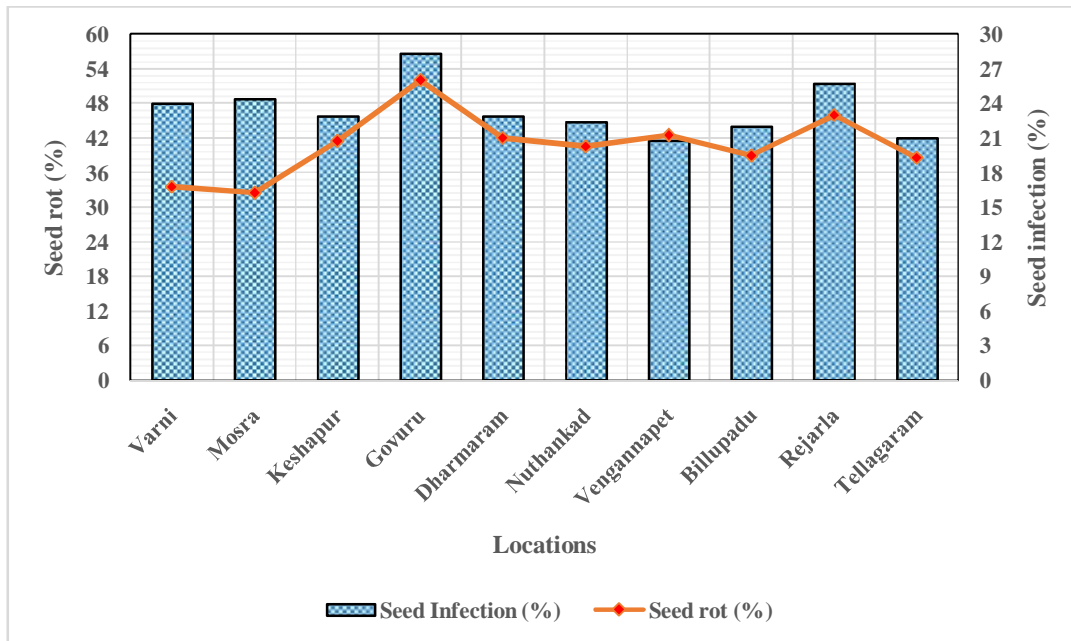
Results and Discussion

The present study revealed that the per cent seed infection (PSI) recorded by the BLB affected paddy seed samples collected from village Govuru of Nizamabad district and Rejarla of Khammam district with 56.63% and 51.36%, respectively, differed significantly from the seed samples of other villages.

Across the locations, by following the agar plate method, the BLB affected seed samples collected from Govuru village of Nizamabad district recorded the highest (56.63%) per cent seed infection and the lowest (41.58%) was observed from the seed samples of Vengannapet village from Khammam district. However, among the seed samples collected from the Nizamabad district, the per cent seed infection recorded by Govuru village was followed by Mosra (48.75%), which was on par with the PSI (48.00%) of seed samples from Varni and significantly differed by the seed samples of Dharmaram (46.00%) and Keshapur (45.75%) villages of Nizamabad district, Nuthankad (44.72%) and Billupadu (44.00%) villages of Khammam district. While the highest (20.75%) per cent seed rot was recorded in the samples collected from Vengannapet village of Khammam and the lowest (11.00%) in Dharmaram of Nizamabad district (Table 1 Fig 1).

The test tube water agar method revealed, the highest significant differences in the *Xoo* infection in the seed samples of Govuru and Rejarla villages, among the ten BLB paddy seed samples collected from different locations. Across the locations, Govuru village of Nizamabad district with 44.50% recorded the highest per cent seed infection and the lowest of 29.50% was observed from the seed samples of Tellagaram village from Khammam district. However, the PSI of 44.50% recorded by the seed samples of Govuru village of Nizamabad district significantly differed from the PSI of 41.25% observed in the seed samples from Rejarla village of Khammam district.

Figure 1 Effect of *Xoo* on per cent seed infection and per cent seed rot by using the agar plate method



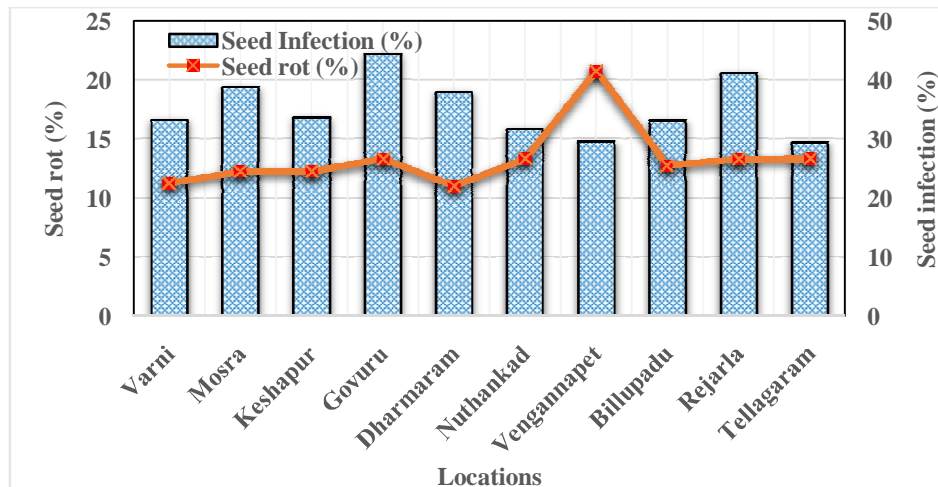
No significant differences in PSI were observed between the seed samples collected from Mosra (38.75%) and Dharmaram (38.00%) villages of the Nizamabad district but they differed significantly with the PSI from seed samples of Vengannapet (29.57%) and Tellagaram (29.50%) villages of Khammam district which are on par with each other. Whereas, the highest (20.75%) per cent seed rot was recorded in the samples collected from Vengannapet village of Khammam and the lowest (11.00%) in Dharmaram of Nizamabad district (Table 1 Fig 2).

Table 1. Seed health status of BLB affected paddy seed samples using agar plate and test tube water agar methods

S.No.	Locations	District	Agar plate method		Test tube water agar method	
			Per cent Seed Infection (PSI)	Per cent Seed Rot (PSR)	Per cent Seed Infection (PSI)	Per cent Seed Rot (PSR)
1.	Varni	Nizamabad	48.00 (43.85)*	16.75 (24.14)	33.28 (35.21)*	11.25 (19.58)
2.	Mosra		48.75 (44.28)	16.25 (23.74)	38.75 (38.49)	12.50 (20.47)
3.	Keshapur		45.75 (42.56)	20.75 (27.07)	33.67 (35.36)	12.25 (20.45)
4.	Govuru		56.63 (48.88)	26.00 (30.63)	44.50 (41.84)	13.25 (21.34)
5.	Dharmaram		46.00 (42.56)	21.00 (27.26)	38.00 (38.05)	11.00 (19.34)
6.	Nuthankad	Khammam	44.72 (41.98)	20.25 (26.70)	31.75 (34.29)	14.00 (21.33)
7.	Vengannapet		41.58 (40.10)	21.25 (27.41)	29.57 (32.88)	20.75 (27.07)
8.	Billupadu		44.00 (41.55)	19.50 (26.18)	33.16 (35.06)	12.75 (20.91)
9.	Rejarla		51.36 (45.72)	23.00 (28.63)	41.25 (39.96)	13.25 (21.33)
10.	Tellagaram		42.00 (40.39)	19.25 (26.01)	29.50 (32.87)	13.50 (21.33)
	Mean		46.85 (43.18)	20.40 (26.77)	35.34 (36.40)	13.35 (21.31)
	CV		3.26	6.41	4.06	5.45
	CD @5%		2.03	2.48	2.13	1.67

*Figures in the parenthesis are angular transformed

Figure 2 Effect of *Xoo* on per cent seed infection and per cent seed rot by using test tube water agar plate method



Further, the results of the component plating method in the study confirmed the recovery of the *Xoo* pathogen in different parts of the seed *i.e.*, seed coat (lemma and palea), embryo and endosperm. The highest *Xoo* recovery was observed from an embryo, which was followed by lemma, palea and endosperm. Among the seed samples of different locations, the palea of seed samples collected from Govuru village, Nizamabad district had the highest 26.75% recovery of *Xoo*. While, the lowest recovery of 14.50% *Xoo* in palea of seed samples was from Billupadu village, Khammam district. The recovery of *Xoo* from the lemma of seed samples collected from different locations stated that, among the locations, the seed samples of Govuru and Varni villages from Nizamabad district recorded the highest 55.44% and lowest 41.67% *Xoo* recovery, respectively. Significant differences were observed in per cent *Xoo* recovery of 46.00% from the seed samples of Mosra, Nizamabad district with the *Xoo* recovery of 43.00%; 43.25%, 42.50% and 42.25% in the seed samples of Keshapur of Nizamabad and Tellagaram Vengannapet and Nuthankad villages of Khammam district.

The *Xoo* recovery from embryos of seed samples collected from the villages revealed that the highest 77.70% per cent of *Xoo* was recovered from the seed samples of village Govuru, Nizamabad district and the lowest 58.89% was noticed in the seed samples of Tellagaram, Khammam district. The highest variation in *Xoo* recovery was observed from the embryos of seed samples collected from different villages of Khammam *viz.*, 75.00% in Rejarla village followed by 72.36% in Vengannapet, 66.63% in Billupadu and 59.52% in Nuthankad which were significantly differed with the seed samples from Varni (70.75%), Mosra (70.25%) and Dharmaram (70.11%) villages of Nizamabad district but were on par among each other.

The data on recovery of *Xoo* pathogen from the endosperm of seed samples reported that the seed samples from Govuru of Nizamabad district reported the highest 17.25% per cent recovery of *Xoo* and the lowest 10.25% in the seed samples from Nuthankad of Khammam district. However, the *Xoo* recovery from village Govuru was followed by 16.25% in the seed samples from Rejarla of Khammam district with no significant differences between them. The per cent recovery of *Xoo* pathogen was observed on par between the seed samples collected from Mosra (13.50%), Keshapur (12.00%) villages of Nizamabad and Vengannapet (12.00%) Tellagaram (13.50%) villages of Khammam but was significantly different with 10.75% of *Xoo* recovery recorded in the seed samples of Billupadu village, Khammam district (Table 2 Fig 3).

Figure 3 Effect of *Xoo* recovery from palea, lemma, embryo and endosperm by using the component plating method

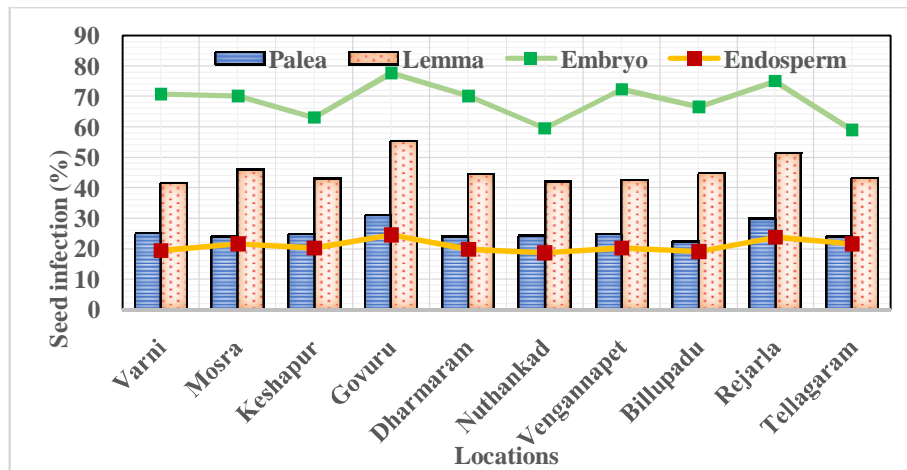


Table 2 Seed health status of BLB affected paddy seed samples using the component plating method

S.No.	Villages	District	Per cent <i>Xoo</i> recovery (%)			
			Palea	Lemma	Embryo	Endosperm
1.	Varni	Nizamabad	18.00 (25.08)*	41.67 (40.25)	70.75 (57.27)	11.00 (19.36)
2.	Mosra		17.00 (23.94)	46.00 (42.70)	70.25 (56.96)	13.50 (21.54)
3.	Keshapur		17.75 (24.91)	43.00 (40.97)	63.00 (52.54)	12.00 (20.25)
4.	Govuru		26.75 (31.13)	55.44 (48.02)	77.70 (61.88)	17.25 (24.52)
5.	Dharmaram		16.50 (23.95)	44.50 (41.83)	70.11 (56.80)	11.50 (19.80)
6.	Nuthankad	Khammam	16.75 (24.15)	42.25 (40.54)	59.52 (50.48)	10.25 (18.65)
7.	Vengannapet		17.50 (24.72)	42.50 (40.68)	72.36 (58.22)	12.00 (20.23)
8.	Billupadu		14.50 (22.37)	44.75 (41.98)	66.63 (54.79)	10.75 (19.13)
9.	Rejarla		25.00 (29.99)	51.50 (45.86)	75.00 (60.03)	16.25 (23.76)
10.	Tellagaram		16.50 (23.95)	43.25 (41.12)	58.89 (50.19)	13.50 (21.54)
	Mean		18.58 (25.41)	45.49 (42.39)	68.42 (55.91)	12.80 (20.87)
	CV		4.51	3.36	2.63	5.49
	CD @5%		1.65	2.05	2.13	1.65

*Figures in the parenthesis are angular transformed

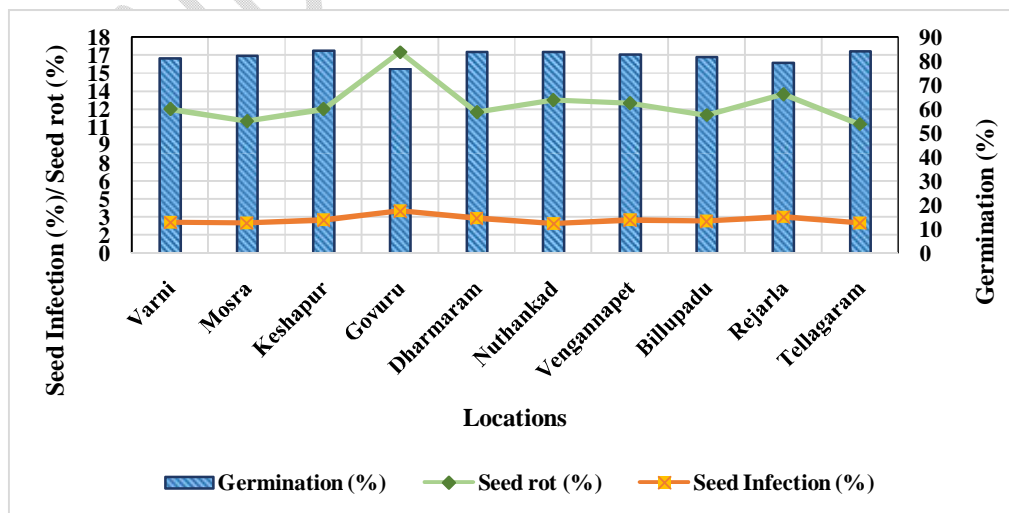
Across the locations, the seed samples collected from Govuru, Nizamabad district recorded the highest recovery of *Xoo* from all the seed components viz., lemma and palea, embryo and endosperm tested.

The results of the paper towel method indicated that, among the ten BLB affected paddy seed samples collected from different locations, all the seed samples have recorded per cent seed germination above Indian Minimum Seed Certification (IMSCS) i.e 80%, except for the seed samples collected from Govuru village of Nizamabad district and Rejarla village of Khammam district which recorded 77.00% and 79.25%, respectively and differed significantly. Across the locations, the BLB affected paddy seed samples collected from Keshapur village with 84.50% recorded the highest per cent seed germination and the lowest 77.00% was observed in the seed samples of Govuru village of Nizamabad district. Further, it was observed that the PSG of these seed samples significantly differed from the PSG of seed samples from Varni (81.50%), Mosra (82.50%) villages of Nizamabad and Billupadu (82.00%) villages of Khammam district.

The present study revealed that, across the locations, the BLB paddy seed samples collected from Govuru village of Nizamabad district recorded significantly highest 17.50% per cent seed infection and the seed samples of Nuthankad village, Khammam district recorded the lowest 12.25% per cent seed infection. Significant differences were observed in per cent seed infection between the seed samples of Dharmaram (14.50%), Nizamabad and Nuthankad (12.25%) Tellagaram (12.75%) villages of Khammam district but were on par with seed samples of Rejarla (15.00%), Khammam.

The data on per cent seed rot stated significant differences among the BLB affected paddy seed samples under study. The highest 16.75% per cent seed rot was observed in the seed samples collected from Govuru and the lowest 10.75% from seed samples of Tellagaram villages, Nizamabad and Khammam districts, respectively. Though, the PSR 13.25% reported in the seed samples of Rejarla village of Khammam district was next to Govuru (16.75%) and was significantly different in recording per cent seed rot. However, the lowest 10.75% PSR recorded by the seed samples in the study from Tellagaram villages was significantly different with 11.00% PSR observed in the seed samples of Mosra village of Nizamabad district (Table 3 Fig 4).

Figure 4 Effect of *Xoo* on per cent seed germination, per cent seed infection and per cent seed rot by using the paper towel method



The BLB affected seeds were ground into a fine powder using a mortar and pestle for direct isolation of *Xoo* from seeds of naturally infected fields. A small amount of sterile water is added to the fine powder and with the help of an inoculation loop, the suspension was streaked on WMA medium. The bacteria streaked plates were then incubated in a BOD incubator at 28 °C for 3-5 days. After 48 h of incubation, yellow colonies appeared which are similar to *Xoo* colonies that were selected and restreaked on WMA plates.

Pure cultures of *Xoo* isolates streaked on WMA and nutrient agar media plates appeared as round, smooth, convex, whitish-yellow to straw yellow later, and opaque. Initially, the colonies appeared as small dots on the 3rd and 4th day and later reached 1-2 mm diameter on the 5th to 7th day with a slow growth rate.

Table 3 Seed health status of BLB affected paddy seed samples using the paper towel method

S.No.	Villages	District	Per cent Germination	Per cent Seed Infection	Per cent Seed Rot
1.	Varni	Nizamabad	81.50 (64.53)*	12.75 (20.91)	12.00 (20.23)
2.	Mosra		82.50 (65.30)	12.50 (20.69)	11.00 (19.36)
3.	Keshapur		84.50 (66.85)	13.75 (21.76)	12.00 (20.26)
4.	Govuru		77.00 (61.36)	17.50 (24.70)	16.75 (24.14)
5.	Dharmaram		84.00 (66.44)	14.50 (22.37)	11.75 (20.04)
6.	Nuthankad	Khammam	84.00 (66.45)	12.25 (20.48)	12.75 (20.91)
7.	Vengannapet		83.00 (65.68)	13.75 (21.76)	12.50 (20.69)
8.	Billupadu		82.00 (64.91)	13.25 (21.33)	11.50 (19.80)
9.	Rejarla		79.00 (63.10)	15.00 (22.75)	13.25 (21.30)
10.	Tellagaram		84.25 (66.64)	12.75 (20.69)	10.75 (19.13)
	Mean		82.23 (65.12)	13.78 (21.74)	12.43 (20.58)
	CV		2.15	5.19	5.66
	CD @5%		2.02	1.63	1.68

*Figures in the parenthesis are angular transformed

The findings of the investigation are in agreement with Ora *et al.*, 2011, who adopted Agar plate and rolled paper towel methods for the detection of seed borne pathogens from cultivated rice varieties in Bangladesh, wherein, in addition to mycoflora the methods could recover *Xanthomonas* sp. from rice seed samples. Also, Singh and Rao (1977) used paper towel method for detecting *Xanthomonas oryzae* in rice seeds. Mondal *et al* (2019) observed a 21 % recovery of *Xoo* colonies from the rice seeds plated on a YDC medium. The results of the study are in confirmity with Mizukami (1961) who reported the presence of

Xoobacterium in husks of rice seed. Srivastava and Rao (1964) stated the presence of the *Xoo* bacterium in the endosperm of the grain and the percentage of less than 1 to 100 per cent carrying of *Xoo* in rice seeds. Kauffman and Reddy (1975) studied in detail the seed transmission of *X. oryzae* in rice and stated that glumes of rice seeds become readily infected by *X. oryzae*.

The pathogenicity studies at 15 days after inoculation revealed expression of water soaked areas along the leaf margins followed by straw-coloured wavy margins and finally withering of seedlings. From artificially inoculated leaves, re-isolation of each isolate was made as per the procedure described in the previous section for completion of Koch postulates.

Conclusions

Of all the seed health testing methods tested, the detection of per cent seed infection of *Xoo* from BLB affected seed samples was high in the Agar plate method followed by the test tube water agar method. Further, the component plating method revealed that, of the different seed components, the per cent recovery of *Xoo* was from embryo followed by lemma, palea and endosperm stating that, *Xoo* had systemic type of infection. The results of the paper towel method indicated that, among the ten BLB affected paddy seed samples collected from different locations, except for the BLB affected seed samples of Govuru village, Nizamabad district and Rejarla village, Khammam district which recorded 77.00% and 79.25%, respectively, all the remaining seed samples have recorded per cent seed germination above Indian Minimum Seed Certification (IMSCS) i.e 80%.

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