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RESEARCH ARTICLE

INTERACTION OF DIFFERENT PATHOGENS ON THE EXTENT OF LESION DEVELOPMENT OF BETELVINE (*PIPERIS BETLE*)

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ABSTRACT

Betelvine (*Piper betle L.)* is the most important cash crop grown almost all over the state of West Bengal, India. The most important betelvine growing districts in West Bengal are Midnapore (East), Howrah, Hooghly, 24-Parganas (South) and Nadia. Besides the above districts, cultivation has now been extended to 24-Parganas (North), Birbhum, Bankura, West Dinajpur and Murshidabad districts too. It is affected by a large number of diseases, which reduces yield and quality of betelvine leaves. Bacterial leaf spot and stem rot disease caused by *Xanthomonas axonopodis pv. betlicola* and *Pseudomonas betel* respectively are gradually become important in West Bengal. *Phoma piperis-betle* is also prevalent in the state. When *X.a. pv. betlicola* and *Ps. Betel* jointly inoculated in same leaf, the size of the lesion become larger as compare to the lesion produced by individual bacterium. Simultaneous inoculation of *X. a. pv. betlicola, Ps. betel* and *P. piperis-betle* did not have pronounced effect in respect to lesion size in leaves. But inoculation of bacteria, 48 hours after inoculation of *P. piperis-betle* greatly increased lesion size.

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INTRODUCTION

Betelvine (Piperbetle) is a perennial dioecious creeper cultivated in India for its leaf since time immemorial. The cultivated betel in India is usually the male plant selected from certain races and consequently does not fruit. This crop belongs to the family Piperaceae and is probably a native of Malaysia. In India, West Bengal, Assam, Karnataka and Tamil Nadu have the highest acreage in terms of cultivation of betelvine. Other important states are Maharastra, Kerala, Andhra Pradesh, Madhya Pradesh, Bihar and Uttar Pradesh.

Besides India, betelvine is also cultivated in Malaysia, Indonesia, Mayanmar, Philippines, Bangladesh, Nepal, Sri Lanka etc. The most important betelvine growing districts in West Bengal are Midnapore (East), Howrah, Hooghly, 24-Parganas (South) and Nadia. Besides the above districts, cultivation has now been extended to 24-Parganas (North), Birbhum, Bankura, West Dinajpur and Murshidabad districts. Common varieties cultivated in India are Bangla, Mitha, Sanchi, Kapoori, Desawari, Khasi and Ghanagnete. Betelvine is cultivated under artificially erected structure known as 'Boroj'. The Boroj is constructed with locally available materials such as bamboo, jute sticks, straw etc. Knowledge gathered from the farmers of West Bengal indicates that Mitha variety required comparatively more shade than Bangla and Sanchi varieties. Thus density of cover or type of covering should vary with various varieties. The matter is very important as it controls the microclimate inside the Boroj as well as the overall performance or yield of vines.

The moist shaded condition prevailing in Boroj favours vine growth but is also congenial for growth of pathogenic fungi and bacteria causing some destructive diseases. A number of leaf spot diseases caused by different fungal and bacterial pathogens (*Colletotrichum capsici, Drechslera rostrata, Cladosporium pipericola, Cercospora piperis, Corynespora cassicola, Phoma piperis-betle, Xanthomonas campestris pv. betlicola(Xanthomonas axonopodis pv. betlicola, Pseudomonas betle*) have been reported which cause damage to betelvine (Mohanti and Mahapatra, 1968; Singh and Joshi, 1974; Maiti *et al*, 1978; Maiti and Sen, 1979; Chattopadhyay and Maiti, 1990; Bardhan *et al*, 2002; Bhattacharya *et al*, 2003).

Among the fungal pathogens of betelvine, *Phoma piperis-betle* is the most important pathogen throughout growth period

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(Hembram and Baskey, 2015). In recent years, bacterial leaf spot (*Xanthomonas axonopodis pv. betlicola & Pseudomonas betle*) is causing considerable damage to betelvine (Bardhan *et al*., 2002; Bhattacharya *et al*., 2003). Incidence of the disease was severe when other pathogens such as *Colletotrichum capsici* and nematodes were involved along with this bacterium (Bhale *et al*, 1985; Acharya *et al*, 1987). Bhattacharya and Khatua (2004) recorded *Phoma piperis-betle* and *Xanthomonas axonopodis pv. betlicola* from the same stem lesion. Maiti *et al*. (1978) reported a leaf spot disease caused by Phoma piperis-betle from West Bengal. Marimuthu and Rabindran (1983) reported bacterial stem rot is a serious menace to betelvine.

Leaf spot disease caused by different pathogens reduces yield and quality of betelvine leaves. After the report of leaf spot disease caused by *Phoma piperis-betle* by Maiti *et al*. (1978), no work has been done on this disease. In the present study, considering the importance of betelvine as a commercial crop in this state and also the importance of bacterial leaf spot disease (*Xanthomonas axonopodis pv. betlicola & Pseudomonas betle*) of betelvine (Bhattacharya and Khatua, 2004) causing much loss to the crop, keeping this view in mind an investigation was undertaken to study effect of interaction *Xanthomonas axonopodis pv. betlicola* and *Pseudomonas betle* with *Phoma piperis-betle*.

MATERIALS AND METHODS

Bacterial culture used

Both the yellow and white colony forming bacteria isolated from infected leaves and stem of betelvines collected from different localities were used for pathogenicity. Yellow colony forming and white colony forming bacteria identified as *Xanthomonas axonopodis pv. betlicola* and *Pseudomonas betle*.

Collection and isolation

Infected tissues (leaf pieces) after surface sterilization with 0.1% HgCl2 for 30 seconds and then washed in sterile distilled water for 2 times under aseptic condition. Finally these were transferred to culture tubes containing 5 ml of Potato Dextorse Chloramphenicol Agar medium. The slants were marked properly and packed in polythene packets and kept in BOD incubator at 280±1$_0$C for development of fungus. Sub-culturing was done at 15 days interval.

Pathogenecity test

Betelvine leaves were inoculated with spore suspension (5X 10^{-6}conidia/ml) of *Phoma piperis-betle* and kept at room temperature. Then the spore suspension was injected into the healthy betelvine leaf by using a syringe. Next the leaf was kept in a polythene packet. Some amount of moist non-absorvent cotton was placed on the packet. Then the packet was filled up with air and was kept in BOD incubator at 28± 10C for growth of fungus. After 48 hours of incubation, circular to irregular spots were seen on the leaf. They were light brown to dark brown in colour. Yellow halo not present in the lesion.

Test plants used

Betelvine plants in Boroj and detached leaves were used as experimental plants per leaves.

Media used

During the course of investigations, the following media were used. For routine cultural work like maintenance of culture and multiplication of bacteria, Potato sucrose peptone agar (PSPA) medium(Peeled potato-200 g, Sucrose-20g, Peptone-5 g, Agar agar-20 g, Distilled water-1000 ml) was used. For other experimental works, Potato dextrose agar (PDA) medium (Peeled potato-200g, Dextrose-20g, Agar-agar-20g, Distilled water-1000 ml was for maintenance of *Phoma piperis-betle* and bioassay of fungicides against *Phoma piperis-betle*. Potato dextrose chloramphenicol agar medium (Peeled potato-200g, Dextrose-20g, Agar-agar-20g, Chloramphericol-100 mg, Distilled water-1000 ml) was used for isolation of *Phoma piperis-betle*.

Effect of interaction of white and yellow colony forming bacteria on the extent of lesion development on leaf

Yellow colony forming bacterium (*Xanthomonas axonopodis* pv. *betlicola*) and white colony forming bacterium (*Pseudomonas betle*) infect same plant or leaf. Both the bacteria have also been isolated from the same leaf spot. Experiment was conducted in the laboratory condition wheather interaction of the bacteria had any effect on the lesion development. Individual leaves were inoculated separately, by two bacteria by injection method. Water soaked area developed after inoculation was more or less same in all the leaves. Fifty percent of inoculated leaves in case of yellow bacterium were painted with white bacterial culture, two days after inoculation, on the lower side of the leaves with the help of a camel hair brush. Application was restricted within water soaked area developed during inoculation. Similarly leaves inoculated with white bacterium were painted with yellow bacterial culture. Leaves were kept in humid condition after application with bacterial culture. Diameter of the individual lesion was measured five days after inoculation.

RESULTS AND DISCUSSION

Betelvine (Piperis betle L.) is an important cash crop grown almost all over the states of West Bengal. It is affected by a large number of diseases (Maity and Sen, 1979). Bacterial leaf spot disease caused by *Xanthomonas campestris pv. betlicola* is gradually becoming a dominating disease in the states. (Bardhan, 2002). In the following pages, the results of investigations on detection of *Xanthomonas campestris pv. betlicola* from diseased and healthy plant tissues and effect of interaction of this bacterium with another one bacterial pathogen (*Pseudomonas betle*) and fungal pathogen (*Phoma piperis-betle*) are presented.

Effect of interaction of white and yellow colony forming bacteria on the extent of lesion development on leaf

Both the bacteria when separately inoculated produced more or

less similar spot with yellow halo. When leaves inoculated with white bacterium subsequently painted with yellow bacterium, the lesion size increased significantly compared to the size of lesion produced by white bacterium only. Similar result obtained when yellow bacterium inoculated leaves were painted with white bacterium. Yellow colony forming bacterium (*Xanthomonas axonopodis* pv. *betlicola*) and *Phoma piperis-betle* infect same plant or leaf. The present experiment was conducted to note whether simultaneous infection of this fungus and bacteria (*Xanthomonas axonopodis* pv. *betlicola* and *Pseudomonas betle*) had any effect on the lesion development.

Table 1 Effect of interaction of white and yellow colony forming bacteria on the extent of betelvine leaf area damage

Leaf inoculated* with			
White bacteria only (*Pseudomonas betle*)	**Painted(lower surface) with yellow bacteria 2 days after inoculation with white bacteria**	**Yellow bacteria only (*X. a. pv. betlicola*)**	**Painted(lower surface) with white bacteria 2 days after inoculation with yellow bacteria**
Diameter of the spot (cm)	Diameter of the spot (cm)	Diameter of the spot (cm)	Diameter of the spot (cm)
1.4	2.0	1.2	1.45
1.2	1.9	0.90	2.4
0.60	2.2	1.2	2.2
1.1	2.2	1.4	1.65
1.5	1.6	1.2	1.9
1.1	1.8	1.3	2.2
1.8	2.0	1.0	2.0
0.90	2.2	0.90	1.5
1.4	2.0	0.40	1.2
1.2	2.4	0.60	2.25
		Average	
1.22	2.03	1.01	1.87
% increase	66.39		85.64

*Inoculated by injection in the vein covering about 0.6 mm diameter water soaked area.

Effect of interaction of Phoma piperis-betle with Xanthomonas axonopodis pv. betlicola and Pseudomonas betle on the extent of lesion development

In this experiment, at first spore suspension of *Phoma piperis-betle* was prepared. (5×10^9 conidia /ml). In a culture tube containing growth of yellow bacteria, distilled water was poured and after stirring, the bacterial suspension was prepared. Now the fungal spore suspension and the bacterial suspension was mixed in the ratio of 1: 1. In the separate set spore suspension was diluted with addition of water (1:1). Next the mixed suspension of fungal spore and bacteria was injected to the healthy betelvine leaf.

On the other hand, some healthy betelvine leaves were inoculated with the fungal spore suspension. All the inoculated leaves were kept in humid condition after inoculation. Diameter of the individual lesion was measured 5days after inoculation. Similarly, another set of experiment was set up with *Pseudomonas betle*. There was marginal increase in diameter of spots (5.8%) when leaves were inoculated with *Phoma piperis-betle* and *Xanthomonas axonopodis* pv. *betlicola* over spot size in leaves inoculated only with the fungus. But there was 18.03% increase in diameter of spots in case of combine inoculation of *Phoma piperis-betle* and *Pseudomonas betle*.

Table 2 Effect of interaction of *Phoma piperis-betel* and *X. a. pv. betlicola* and *Pseudomonas betle* on the extent of betelvine leaf area damage

Leaf inoculated* with			
Only fungus	**Fungus and yellow bacteria**	**Fungus only**	**Fungus and white bacteria**
Diameter of the spot (cm)	**Diameter of the spot (cm)**	**Diameter of the spot (cm)**	**Diameter of the spot (cm)**
1.90	2.50	1.80	2.60
2.50	2.75	2.90	2.10
2.80	2.70	1.80	2.80
1.90	3.50	2.50	2.10
2.80	3.50	2.40	2.90
2.50	2.60	1.90	3.10
3.10	2.70	2.50	3.10
3.30	2.50	2.40	2.52
3.00	3.50	1.60	2.80
3.20	3.51	2.50	2.30
	Average		
2.7	3.0	2.2	2.6
% increase	10.22		18.03

Inoculated by injection in the vein covering about 0.6 mm diameter water soaked area.

Prominent yellow halo developed around the brown colour lesion when leaves were inoculated with *Phoma piperis-betle* plus *Pseudomonas betle* or *Phoma piperis-betle* plus *Xanthomonas axonopodis* pv. *betlicola*. But the halo was not prominent when the leaves were inoculated only with the fungus, *Phoma piperis betle*.

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References

1. Acharya, A., Dash, S.C. and Padhi, N.N. 1987. Pathogenic association of *Meloidogyne incognita* with *Sclerotium rolfsii* and *X. betlicola* on betelvine. Indian Journal of Nematology. 17 (2) : 196-198.
2. Bardhan, S., Bhattacharya, R. and Khatua, D.C. 2002. Present status of bacterial disease of betelvine in West Bengal. Proceedings of National Seminar on Frontier studies in microbes and host-microbe interactions, organized by Indian Mycological Society, Kolkata, on February 2-3, 2002: 43 (abs).
3. Bhale, M. S., Chaurasia, R. K, and Nayak, M. L. 1985. Association of Colletotrichum capsici with *Xanthomonas campestris pv. betlicola* incitant of leaf spot of betelvine (Piper betle). Indian Phytopathology 38 (3) : 365-366
4. Bhattacharya, Rana; Bardhan, Saswati and Khatua, D. C. 2003. bacterial disease of betelvine - nature of pathogens. Accepted for presentation in National Symposium in current Trends in Research on Microorganisms. Organized by Indian mycological society, Kolkata, on February 15-16, 2003: (abs).
5. Bhattacharya, R. and Khatua, D.C. 2004. Bacterial disease of betelvine in West Bengal- nature of pathogens. Indian Phytopathol. 57(3):357(abs).

6. Chattopadhyay, S.B. and Maiti, S. 1990. Diseases of betelvine and spices. ICAR, New Delhi pp.1-32.
7. Hembram, S. and Baskey, S. 2015. In vitro evaluation of some chemicals against *Phoma piperis-betle* causing leaf spot disease of Betelvine. International Journal of Sciences & Applied Research. 2(10): 31-35.
8. Maiti, S., Khatua, D.C. and Sen, C. 1978. A new leaf spot disease of betelvine. Indian J. Mycol. Pl. Pathol. 8: 217.
9. Maiti, S. and Sen, C. 1979. Fungal diseases of Betelvine. PANS. 25: 150-57.
10. Mohanty, N.M. and Mahapatra, P.K. 1968. A new leaf spot disease of betelvine caused by *Corynespora cassicola*. Indian Phytopathol. 21:275-280.
11. Singh, B. and Joshi, L.K. 1974. Studies on diseases of betelvine (Piper betle L.) in Jabalpur (Madhya Pradesh). New leaf spots caused by *Drechslera rostata* and *Cochliobolus spicifer*. Punjabrao Krishi Vidyapith Res. J. 2(2):140.

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