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# *In vitro* evaluation of some chemicals against *Phoma piperis-betle* causing leaf spot disease of Betelvine

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## Abstract

Betelvine (*Piper betle L.)* 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. Diseases are one of the major constraints behind the poor productivity of betelvine. Among the biotic stresses of betelvine, *Phoma piperis-betle* is the most important pathogen throughout growth period. Application of fungicide is an important aspect of plant disease management but less application of fungicides is desirable. An *in vitro* study was therefore undertaken to examine the relative efficacy of six fungicides on the growth of *P. piperis-betle* following standard poisoned food technique at different concentration. Carbendazim completely inhibited the growth of *P. piperis-betle* only at 10 ppm, followed by mancozeb with 100 percent inhibition in 50 ppm concentration. The growth of inhibitions of 100 and 82.00 percent were separately observed in 100 ppm of Zineb and Ziram respectively, in comparison to untreated control/check. Growth inhibitions of 52.88 and 37.88 percent were recorded in 500 ppm of copper oxychloride and metalaxyl. Thus, among all the six fungicides tested, carbendazim and Mancozeb have the great potentiality in comparison to copper oxychloride and zineb in the management of *Phoma* leaf spot disease of betelvine.

**Keywords:** Betelvine, leaf spot, *Phoma piperis-betle*, fungicide

## Introduction

Betel vine (*Piper betle L)* is known by its many names across the country and abroad. It belongsto *piperaceae* family and is a perennial climber cultivated for its leaf. It is a shed loving plant and originated from Malaysia according to De Cando. Historically, the word pan in Hindi and other Indian languages is probably a derivative of the Sanskrit word 'pan' meaning leaf. The fresh crushed leaves are used as antiseptic for cuts and wounds. It is also good for the respiratory system and is used in treatment of bronchitis, cough and cold (Chopra et al, 1958). In India it is grown over an area of about 50,000 hectare. The annual turnover of betel vine is estimated at Rs. 10,000 million. 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 structures known as '*Boroj'*. The moist and dappled conditions prevailing in *Boroj* favour vine growth and are also congenial for development of several fungal and bacterial diseases (Maiti and Sen, 1979; Maiti, 1994; Maiti and Shivashankara, 1998).

Among the several diseases of betelvine, 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). 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. 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 leaf spot disease of betelvine (Bhattacharya and Khatua, 2004) causing much loss to the crop, an investigation was undertaken to study sensitivity of these pathogens towards different fungicides was studied.

## Materials and methods

### Media used

For preparation of Potato dextrose agar (PDA) medium, 200g of peeled potato was thoroughly washed and cut into pieces and boiled in 500 ml of distilled water. The decoction was filtered. Then 20g dextrose was mixed with the extract. In a separate beaker, agar-agar was melted in boiling distilled water (500ml) and mixed with the extract and excess water was added to make the volume upto 1 litre.

The medium was poured in conical flask @200 ml/flask, plugged with nonabsorbent cotton and sterilized at 121°C temperature under 15lbs/sq inch pressure for 15 minutes in an autoclave. This medium was for maintenance of *Phoma piperis-betle* and bioassay of fungicides against *Phoma piperis-betle*.

Method of preparation Potato dextrose chloramphenicol agar medium was same as PDA medium. But here, before sterilization, 100 mg chloramphenicol was mixed in the medium to avoid bacterial contamination. This medium was used for isolation of *Phoma piperis-betle*.

### Collection and isolation

Infected tissues (leaf pieces) after surface sterilization with 0.1% $HgCl_2$ 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 $28^0 \pm 1^0$C for development of fungus. Sub-culturing was done at 15 days interval.

**Pathogenecity test**

Betelvine leaves were inoculated with spore suspension ($5\times 10^6$ 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 $28^0 \pm 1^0$C 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.

**Bioassay of fungicides**

Six different fungicides namely Bavistin (Carbendazim 50% WP), Indofil M-45 (Mancozeb), Indofil Z-78 (Zineb 75% WP), Ziram 27% SC, Krylaxyl 35 (35% Metalaxyl) and (Copper oxychloride 50%WP) were tested against *Phoma piperis-betle* at different concentrations (500, 200, 100, 50, 20, 10, 5, 3 ppm) *in vitro* condition following poisoned food technique. The principle in this technique was to poison the nutrient (medium) with a fungitoxicant and then the test fungus was grown on such a medium. In this technique either a solid (Agar) or a liquid medium can be used.

PDA medium was prepared in conical flasks and sterilized by autoclaving at 15 p.s.i. for 15 minutes. To this medium, requisite quantity of the fungicide was added so as to get the desired concentration. A series of concentration were thus prepared. The medium was then poured into sterilized petriplate and allowed to solidify. A small disc (0.7 cm) of the test fungal culture (ten day old) was cut and transferred into the solidified PDA medium in petriplates under aseptic condition. Suitable checks were kept where the culture disk were allowed to grow on the PDA medium without fungicide. The colony diameter was measured after 14 days. The colony diameter compared with check was taken as a measure of fungitoxicity. Relative fungitoxicity was measured using Vincent's (1927) formula-

$$I = \frac{C - T}{C} \times 100$$

Where, I = Inhibition of mycelial growth, C = Growth in control, T = growth in treatment

**Results and discussion**

Among the six fungicidal compounds evaluated against *Phoma piperis-betle* all were found inhibitory to the fungus with varied degree of inhibition. The results indicated that out of six chemicals tested by employing poison food techniques on mycelial growth of *P. piperis-betle*. Carbendazim completely inhibited the growth of *P. piperis-betle* only at 10 ppm, followed by mancozeb with 100 percent inhibition in 50 ppm concentration (Table-1).

Similar results were reported by Jamaluddin *et al.* (1988) as Bavistin and Mancozeb was found effective in controlling *Phoma* leaf spot disease. Carbendazim were also reported to be highly effective in controlling *Phoma* (Parisi, *et al.*,1999; Patil, *et al.*, 2010; Saju, *et al.*, 2011). The growth of inhibitions of 100 and 82.00 percent were separately observed in 100 ppm of Zineb and Ziram respectively, in comparison to untreated control/check.

**Table 1. Screening of some chemicals against *Phoma piperis-betle* following standard poisoned food technique.**

| Fungicides | Concentration(ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 500 | 200 | 100 | 50 | 20 | 10 | 5 | 3 |
| | Diameter of inhibition zone in cm | | | | | | | |
| Copper oxychloride 50%WP | 52.88 (46.66) | 16.33 (23.83) | 10.88 (19.26) | 7.00 (15.34) | 5.00 (12.91) | 2.70 (9.65) | 0.00 (4.05) | 0.00 (4.05) |
| Ziram 27%SC | 91.11 (72.66) | 84.66 (66.95) | 82.00 (64.90) | 79.11 (62.81) | 57.77 (49.47) | 44.44 (41.81) | 26.44(30.94) | 14.22 (22.15) |
| Zineb 75%WP | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 19.00 (25.84) | 14.33 (22.24) | 10.77 (19.15) | 0.00 (4.05) | 0.00 (4.05) |
| Mancozeb 75%WP | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 38.66 (38.45) | 25.55 (30.37) | 22.55 (28.35) | 16.88 (24.26) |
| Carbendazim 50% WP | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 61.22 (59.71) | 46.88 (51.49) |
| Metalaxyl 35%WS | 37.88 (37.98) | 24.30 (29.54) | 18.33 (25.34) | 10.77 (19.15) | 7.88 (16.30) | 2.88 (9.70) | 0.00 (4.05) | 0.00 (4.05) |
| SEm± | 0.27 | 0.19 | 0.20 | 0.26 | 0.21 | 0.36 | 0.14 | 0.16 |
| CD at 5% | 0.78 | 0.56 | 0.59 | 0.77 | 0.63 | 1.06 | 0.43 | 0.47 |

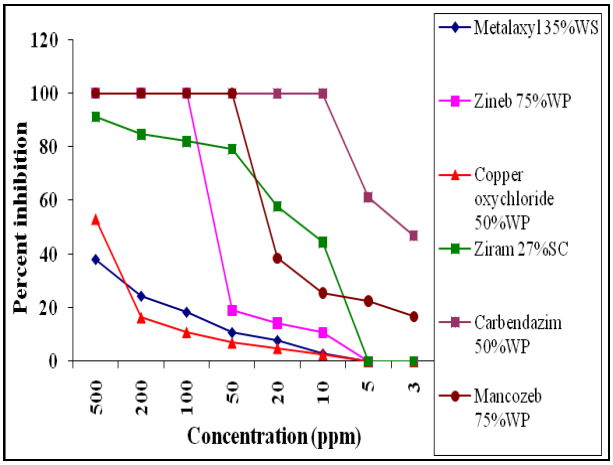


**Fig. 1. Extent of sensitivity of *Phoma piperis -betle* towards different fungicide.**

Increased mycelial inhibition due to increasing concentration of fungicide was observed under poison food technique method. Growth inhibitions of 52.88 and 37.88 percent were recorded in 500 ppm of copper oxychloride and metalaxyl. Thus, among all the six fungicides tested, carbendazim and Mancozeb have the great potentiality in comparison to copper oxychloride and zineb in the management of *Phoma* leaf spot disease of betelvine (Fig.1).

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**References**

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).

Bhattacharya, R. and Khatua, D. C. 2004. bacterial disease of betelvine in West Bengal- nature of pathogens. *Indian Phytopathology* 57(3):357(abs).

Bhattacharya, R., Bardhan, S. 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).

Chattopadhyay, S. B. and Maiti, S. 1990. Diseases of betelvine and spices. ICAR, New Delhi pp. 1-32.

Jamaluddin, Dadwal, V.S. and Soni K.K. 1988. Some new and noteworthy diseases of *Gmelina arborea* Roxb. from Madhya Pradesh. *Journal of Tropical Forestry* 4 (3) 297-299.

Maiti , S., Khatua, D. C. and Sen, C. 1978. A new leaf spot disease of betelvine. *Indian .J. Mycol. Pl. Pathol*. 8: 217.

Maiti, S. 1994. Diseases of betelvine. In: Advances in horticulture. Vol. 10. Plantation and spice crops. Part 2. (Eds. KL Chadha, P Rethinam). Malhotra Publishing House, New Delhi, India. pp. 1085-1097.

Maiti, S. and Sen, C. 1979. Fungal diseases of betel vine. PANS 25(2):150-157.

Maiti, S. and Shivashankara, K.S. (1998). Betelvine research highlights (1981–1997). All India Coordinated Research Project on Betelvine, IndianInstitute of Horticultural Research, Bangalore, India 21pages.

Mohanty, N. M. and Mahapatra, P.K. 1968. A new leaf spot disease of betelvine caused by *Corynespora cassicola*. Indian Phytopathology 21:275-280.

Parisi, J. J. D.; Menten, J. O. M.; and Martins, M. C. 1999. Sensitivity of *Phoma sojae* and *Phoma phaseoli* f.sp. *meridionalis* to fungicides *in vitro* and *in vivo*. *Fitopatologia Brasileira* 24 (1) 25-30.

Patil, V.A., Mehta, B.P. and Deshmukh, A. J. 2010. Field evaluation of different fungicides against *Phoma* leaf spot disease of indian bean. International Journal of Pharma and Bio Sciences 1(2): 1-5.

Saju, K. A., Deka, T. N., Sudharshan, M. R., Gupta, U. and Biswas, A. K. 2011. Incidence of *Phoma* leaf spot disease of large cardamom (*Amomum subulatum* Roxb.) and in vitro evaluation of fungicides against the pathogen. *Journal of Spices and Aromatic Crops.* 20 (2): 86–88.

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.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 59:850.