

In Vitro Evaluation of Botanicals, Bio-Agents and Fungicides against Leaf Blight of *Etlingera linguiformis* Caused by *Curvularia lunata* Var. *Aeria*

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Abstract

The present study was attempted with the objective of screening the potential antifungal activities of eight fungicides, nine plant extracts and five bio-agents *in vitro* against leaf blight of *E. linguiformis* caused by *C. lunata* var. *aeria*. Mancozeb at 0.3% (97.37% inhibition) was found to be significantly superior among the non-systemic fungicides evaluated at three concentrations (0.1, 0.2 and 0.3%). Among the systemic fungicides, metalaxyl showed 98.48% inhibition of mycelial growth at 0.3% concentration followed by carbendazim (95.25% inhibition at 0.3%). Among the plant extracts, *Millettia pachycarpa* root extracts (55.78) at 10 per cent was superior followed by *Acorus calamus* with 53.40% inhibition at 10 per cent. Among the bio-agents tested, *Trichoderma harzianum* showed maximum inhibition of 68.85 per cent inhibition. The study indicated that suitable integration of more efficient eco-friendly treatments like bio-agents and botanicals with lesser use of fungicides may provide effective management of the disease.

Keywords: *Etlingera linguiformis*; *Curvularia lunata* var. *aeria*; Leaf blight; Fungicides; Bio-agents; Plant extracts

Introduction

Etlingera linguiformis (Roxb.) R.M.Sm of Zingiberaceae family is an important indigenous medicinal and aromatic plant found in Nagaland. The plant species was identified from Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (BSI/ERC/2014/Plant identification/883) has been deposited in the herbarium of the Botanical Survey of India, Shillong. It grows well in warm climates with loamy soil rich in humus. The entire plant is aromatic. The rhizome of the plant is used medicinally to treat sore throat, stomachache, rheumatism and respiratory complains etc. The aromatic rhizome of *E. linguiformis* contains an essential oil used in perfumery. Young leaves and shoots are used as leafy vegetables. Rhizomes are used as medicine to cure jaundice [1]. Recently, severe symptoms typically of *Curvularia* leaf blight were observed. The disease is mainly a foliar disease. Leaf blight of *Curvularia* species have been reported as casual agents of leaf spots and leaf blight [2]. Initial symptoms of the disease are small brown water soaked flecks appears on the upper leaf surface with diameter ranging from 0.5 to 3 cm which later coalesced to form dark brown lesions with a well-defined border. Lesions often merged to form large necrotic areas, covering more than 90% of the leaf surface, which contributed to plant death. The pure culture of the fungus was examined under microscope and identified as *Curvularia lunata* var. *aeria*. The morphological authenticity of the isolated fungus was confirmed from the Indian Type Culture Collection, Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi (ITCC Accession No. 7895.10). Further molecular identification was done through DNA sequencing by using primers ITS4 and ITS5 from the Microbial Type Culture Collection & Gene Bank at the Institute of Microbial Technology (IMTECH), Chandigarh, India (Accession No.

MTCC11875). The disease significantly reduces the number of functional leaves. Late in the disease progression, stems and rhizomes were also affected thereby reducing oil yield and quality. Leaf blight of *Java citronella* (*Cymbopogon winterianus* Jowitt.), caused by *Curvularia* spp., and was found to cause a dramatic change in oil yield and its constituents [3]. It was also observed that *Curvularia* leaf blight infection increased with the age of the crop. Therefore, in view of the magnitude of damage caused by the fungus on this important plant, the present investigation was undertaken to screen out the most efficient fungicides, botanicals and bioagents against *C. lunata* var. *aeria* for field management of the disease.

Materials and Methods

The present investigation on *in vitro* bioassay of different fungicides, botanicals, and bioagents was carried out at the Department of Plant Pathology, School of Agricultural & Rural Development, Nagaland University during 2011-2012. Eight fungicides consisting of four systemic and four non-systemic were assayed for their efficacy against *C. lunata* var. *aeria* under *in vitro* condition. Each of the fungicides were tested at 0.1, 0.2, 0.3 per cent concentrations. The poisoned food technique was adopted for *in vitro* testing of fungicides [4]. The calculated quantities of fungicides were thoroughly mixed in the medium before pouring into Petriplates so as to get the desired concentration of active ingredient of each fungicide separately. Twenty ml of fungicide amended medium was poured in each of 90 mm sterilized Petriplates and allowed to solidify. The plates were inoculated centrally with 8 mm disc of 10 days old young sporulating culture of *C. lunata* var. *aeria*. Controls without fungicides were also maintained. The experiment was conducted in Completely Randomised Design (CRD) with three replications in each treatment. The inoculated petriplates were incubated at 25±2°C. The colony diameters were measured after 10 days when the control plates were full of fungal growth. Per cent inhibition of growth was calculated by using formula given by Vincent [5].

$$I = [(C - T) / (C)] \times 100$$

Where, I=Per cent inhibition; C=Colony diameter in control; T=Colony diameter in treatment

Plant tissues was grounded using pestle and mortar by adding equal amount of sterilized distilled water (1:1 w/v). The extract was filtered through muslin cloth. The supernatant was taken as standard plant extract solution (100%). Further, the extract was diluted by adding sterilised water to get 5, 7.5 and 10 per cent concentrations respectively. The plant extracts were subjected to heating to 50°C in water bath to avoid contamination and then incorporated into PDA medium by transferring 2 ml of each type of plant extracts into a Petridish containing 20 ml melted warm PDA medium and gently shaken for thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated aseptically with *C. lunata* var. aeria by transferring 8mm diameter agar disc of 10 days old culture of the pathogen to the center of PDA medium in Petridish. Three replications were maintained for each treatment. The basal medium (PDA) without any plant extract was served as control. All the inoculated Petridishes were incubated at 25 ± 2°C. The radial growth of the test fungus in the treated plates was measured when the pathogen growth touched the periphery in the control Petridishes. The per cent inhibition of fungal growth was estimated by using the formula given by Vincent [5].

Five bioagents, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii*, *Pseudomonas fluorescens* and *Bacillus subtilis* at 10⁸ spores/ml were evaluated against *C. lunata* var. aeria by dual

culture technique. The bio-agents as well as the test pathogen were inoculated equidistant on PDA medium aseptically and incubated at 28 ± 2°C. Three replications were maintained along with untreated control. After obtaining the maximum growth in the control, the zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organisms. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula suggested by Vincent (Figures 1-5) [5].

Statistical analysis

The statistical analysis of obtained results was carried out by using SPSS (Statistical Package for the Social Sciences) Version 16.0 software. Per cent data were transformed to arc sine values and analyzed statistically.

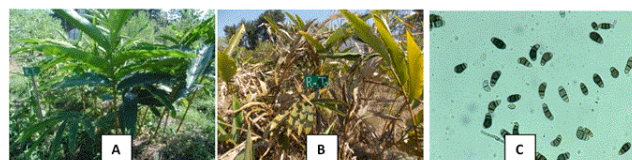


Figure 1: Showing the following- Plate 1: A-Healthy Plant, B-Leaf blight infection, C- *Curvularia lunata* var. aeria

Sl.No	Treatments	Per cent inhibition Concentration (%)			
I	Non systemic fungicides	0.001	0.002	0.003	Mean
1	Captan	29.86	44.96	76.56	50.46
		-33.1	-42.11	-61.06	
2	Chlorothanil	24.13	40.65	61.82	42.2
		-29.41	-39.61	-51.84	
3	Copper oxychloride	33.16	52.79	91.6	59.18
		-35.15	-46.6	-73.18	
4	Mancozeb	43.79	64.62	97.37	68.59
		-41.43	-53.5	-80.77	
II	Systemic fungicides				
1	Carbendazim	51.32	76.78	95.25	74.45
		-45.76	-61.21	-77.47	
2	Hexaconazole	41.49	53.94	90.34	61.92
		-40.09	-47.26	-71.99	
3	Metalaxyl	60.87	79.87	98.48	79.74
		-51.29	-63.36	-83.01	
4	Mycobutanil	25.16	41.08	51.06	39.1
		-30.1	-39.86	-45.6	

	CD (p=0.05)	3.57
	S.Em±	4.72
	CV%	3.65

Table 1: *In vitro* evaluation of fungicides against *C. lunata* var.aeria, *E. linguiformis* leaf blight pathogen. *Figure in parenthesis is arcsine transformations.

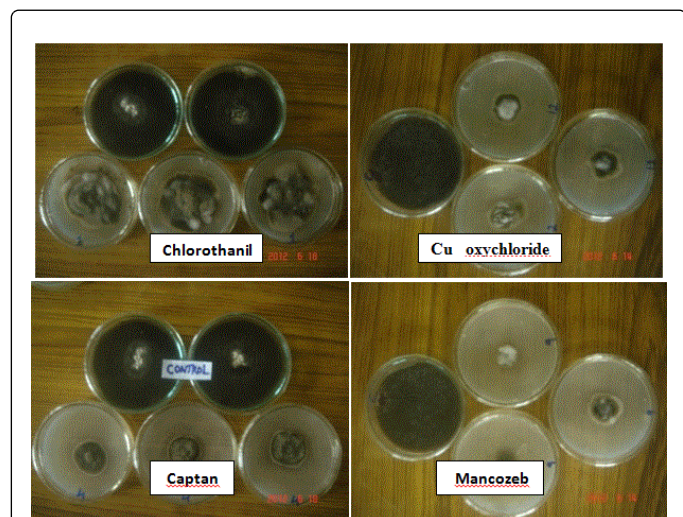


Figure 2: Non-Systemic fungicide

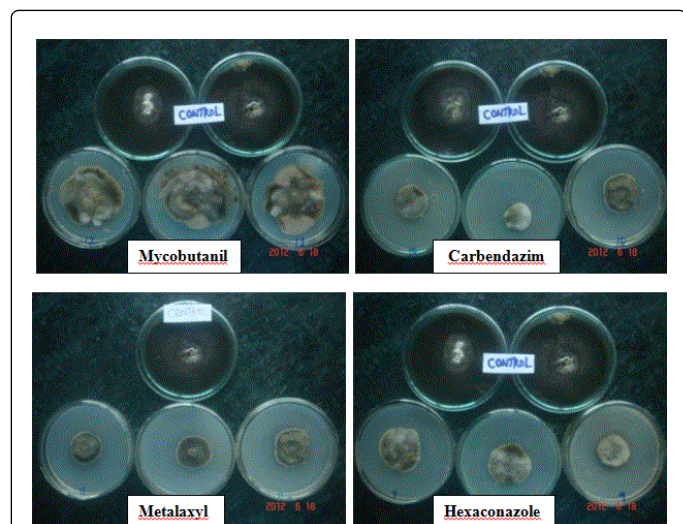


Figure 3: Systemic fungicides

Results and Discussion

In vitro evaluation of provides useful and preliminary information regarding efficacy of fungicides. Plant extracts and bio-agents against pathogen within a shortest period of time and therefore, serves as a guide for field testing. Chemical control measures have been tested and found effective in the control of diseases [6,7]. Certain protective

fungicides although hazardous to environment are still used for the control of fungal diseases [8].

In the present investigation, four systemic fungicides and four non-systemic fungicides were tested at three concentrations (Tables 1-3). Among the non-systemic fungicides tested, mancozeb and copper oxychloride was found to be most effective in inhibiting the mycelia growth at all three concentrations (0.1, 0.2 and 0.3%) with inhibition of 97.37% and 91.60% respectively.

S.No	Plant extracts	Per cent inhibition of mycelia growth Concentrations (%)				
		Parts used	5	7.5	10	Mean
1	<i>Acorus calamus</i>	Rhizome	29.61	45.21	53.4	42.74
			-32.95	-42.3	-47	
2	<i>Allium sativum</i>	Bulb	22.82	30.58	44.11	32.5
			-28.51	-33.6	-41.6	
3	<i>Azadirchta indica</i>	Leaf	17.7	27.82	42.19	28.23
			(22/43)	-31.8	-40.5	
4	<i>Curcuma caesia</i>	Rhizome	27.95	42.55	47.85	39.45
			-31.91	-40.7	-43.8	
5	<i>Curcuma longa</i>	Rhizome	13.15	29.86	37.33	26.78
			-21.2	-33.1	-37.7	
6	<i>Lawsonia inermis</i>	Leaf	19.41	31.11	38.32	29.61
			-26.12	-33.9	-38.2	
7	<i>Millettia pachycarpa</i>	Root	34.03	47.28	55.78	
			-35.58	-43.4	-48.3	45.69
8	<i>Oroxylum indicum</i>	Bark	17.47	23.68	33.55	
			-24.68	-29.1	-35.4	24.9
9	<i>Zingiber officinale</i>	Rhizome	13.13	30.34	37.42	
			-21.21	-33.4	-37.7	26.96
CD (p=0.05)			3.29			
S.Em±			5.75			
CV%			7.27			

Table 2: *In vitro* evaluation of different plant extracts against mycelia growth of *C. lunata* var.aeria. * Figure in parenthesis is arcsine transformations.

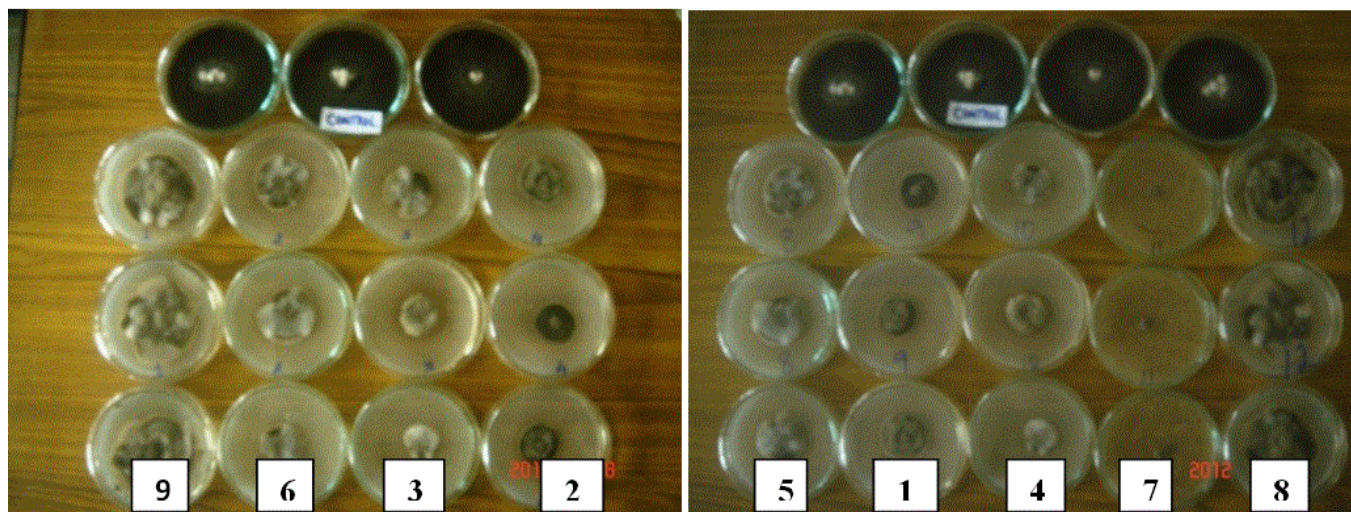


Figure 4: Plant extracts

Sl.No	Biocontrol agents	Per cent inhibition of mycelia growth
1	<i>Trichoderma harzianum</i>	68.85 (56.10)
2	<i>Trichoderma viride</i>	57.82 (49.50)
3	<i>Trichoderma koningii</i>	48.35 (44.05)
4	<i>Pseudomonas fluorescens</i>	51.36 (45.78)
5	<i>Bacillus subtilis</i>	30.32 (33.39)
	CD (p=0.05)	5.29
	S.Em±	8.47
	CV%	5.67

Table 3: *In vitro* evaluation of biocontrol agents against mycelia growth of *C. lunata* var. aeria, the causal agent of leaf blight of *E. linguiformis*. *Figure in parenthesis are arcsine transformations.

It was followed by captan (76.56%) at 0.3 per cent. Pawar et al. [9] also reported that eight fungicides were screened against *C. lunata* and *C. pallescens*. Out of them, mancozeb (0.2%), tricyclazole (0.1%) and campainion (mancozeb + carbendazim) (0.25%) were found effective against *C. lunata*. Jackson [10] reported that blight disease can be controlled by spraying with copper fungicides. Singh et al. [11] reported that mancozeb was effective against *C. lunata* var. aeria. The least effective among the non-systemic fungicides was chlorothanil (61.82% inhibition). Among the systemic fungicides, metalaxyl and carbendazim were found to be highly effective in inhibiting the growth of *C. lunata* at 0.3% concentrations (98.48% and 95.25% inhibition) followed by hexaconazole (90.34%) at 0.3 per cent. Adejumo [12] reported that the use of fungicides such as copper and copper metalaxyl-based compounds is the most reliable and popular with farmers because of the quick and effective action. Efficacy of these fungicides was previously reported by Sumangala et al. [13] and Jackson [10]. Mycobutanil was found to be the least effective (51.06% inhibition) among the systemic fungicides evaluated.

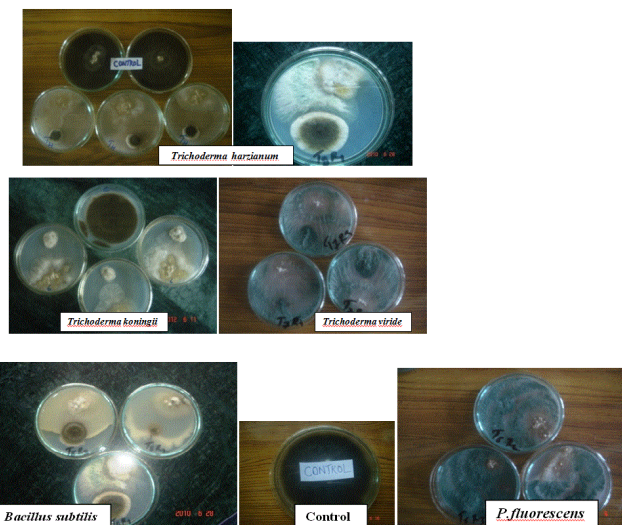


Figure 5: Biocontrol agents

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [14]. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory [15,16]. Plant extracts show antifungal activity against a wide range of fungi [17]. Plants in their natural state possess a relative stable biological balance with microbes on their surface. In the present investigation, though complete inhibition of the fungus was not observed in any of the nine botanicals used, considerable amount of inhibition of growth was noticed in some of the botanicals. Root extracts of *M. pachycarpa* was found to be most effective at all the three concentrations 5% (34.03%), 7.5% (47.28%), 10% (55.78%) followed by *A. calamus* rhizome extract (53.40% inhibition) at 10 per cent. The fungicidal effects of *Acorus calamus* (sweet flag) has already been investigated by Parinthawong

[18]. Bulb extracts of *Allium sativum*, *Azadirachta indica*, *Lawsonia inermis* and *Azadirachta indica* are also effective to some extent. Later, Choi et al. [19], Bandara et al. [20] reported *Acorus calamus*, *Zingiber zerumbet* and *Curcuma longa* to possess several important biological activities including antifungal. Crude extracts of *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, tested *in vitro* showed significant antifungal activity against *Curvularia* sp. [21]. Significant inhibition of *Dreschlera graminea*, *Curvularia lunata*, *Aspergillus fumigatus* and *Candida albicans* were observed with petroleum ether and benzene fractions of the leaf of the leaf of *Lawsonia inermis* [22]. Pawar et al. [9] reported that Garlic (*Allium sativum*) completely inhibited the mycelial growth of both pathogenic fungi followed by *Azadirachta indica*.

Biological control through the use of antagonistic microorganisms is a potential non-chemical means of controlling plant disease by reducing inoculum levels of the pathogens. Such a management would help in preventing the pollution and also health hazards. In the present investigation, the antagonistic effect of different bioagents assessed against *C. lunata* var. aeria by dual culture technique, *T. harzianum* has inhibited maximum growth of fungus with 68.85% followed by *T. viride* (57.82%), and *P. fluorescens* (51.36%). *Bacillus subtilis* was found to be the least effective (30.32%). The inhibitory effect of these bio-agents may be due to competition and antibiosis. Pawar et al. [9] reported that *T. harzianum* and *T. viride* significantly inhibited growth of *C. lunata in vitro*. The present investigations are in agreement with Sumangala et al. [13], who found effectiveness of *Trichoderma* sp. against *C. lunata*. This could be obviously due to several possibilities of existence of microbial interactions such as stimulation, inhibition, mutual intermingling of growth of antagonistic isolate over test pathogen etc. have been enumerated by many workers [23-25]. The antagonistic activities of *T. harzianum* against several pathogenic fungi have also been reported by many workers [26-28]. The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde compound [29]. Sumangala et al. [13] reported that *B. subtilis*, *T. viride* and *T. harzianum* were found effective in inhibiting the radial growth of fungus.

Conclusion

The present study revealed that Metalaxyl and mancozeb @ 0.1 per cent found most effective in inhibiting the growth of *C. lunata* var. aeria. Carbendazim and Hexaconazole both at 0.1 per cent were also found equally effective which can be used as an alternative to mancozeb and metalaxyl. Among the bioagents, *Trichoderma harzianum* was found to be most effective in inhibiting the mycelial growth. *Millettia pachycarpa* and *Acorus calamus* extracts at 5.0 per cent showed maximum growth inhibition among the plant extracts. Thus the study indicated that suitable integration of more efficient eco-friendly treatments like bio-agents and botanicals with lesser use of fungicides may provide a better management of the disease.

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