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Aerofungi over cabbage field in Moreh - A border town of India and Myanmar (Burma)

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The present paper deals with aerofungi over cabbage field in Moreh - a border town of India and Myanmar. Air samplings were conducted for two years (January 2012 to December 2013) by employing Andersen two stage air sampler . Sample collections were done during the day time. So far, 37 fungal types were identified .Among them *Aspergillus fumigatus* occupied the highest (15.4 %) followed by *Gliocladium penicilloids* (13.7%), and *Aspergillus niger* (13.24 %) and the lowest was *Drechelera* sp. (0.18%). *Gliocladium penicilloids* was found as causal organism for yellowing and shriveling of cabbage leaves. Attempts to control wilt disease of cabbage, caused by *Gliocladium penicilloids* by using phytoextracts and systemic fungicides were made. Among phytoextracts used *Solanum anguivii* followed by *Melia azedarach* and *Allium tuberrosum* were found little effective to *Gliocladium penicilloids*. Whereas among the three systemic fungicides used Thiophanate methyle followed by Carbendazim proved to be fungistatic to *Gliocladium penicilloids*.

Key words: Aerofungi, Moreh, Andersen sampler, *Gliocladium penicilloids*

INTRODUCTION

Moreh (184.8 sq.km area, lies between 24Ú13'5"-24Ú26' N latitude and 94Ú23'51"E, 276m-888 m asl) is an ethnic Kuki and Meitei with a sizeable population of Tamil, Nepali and Muslims inhabited town in Chandel district of Manipur state. It is a fast developing and an important trade point in India bordering with Myanmar. Nowadays, Moreh is known as the commercial hub of the state being India's Gateway to South-East Asia. Like other hill districts of Manipur, Chandel is also rich with various kinds of orchids, ornamental plants etc.

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The genus *Gliocladium* belongs to the sub-family Gliosporae, family Moniliaceae, order Moniliales, sub-class Hyphomycetes, class Deuteromycetes and kingdom Mycota. This genus is often characterized as a counterpart of *Penicillium* with slimmy conidia (Gilman, 1956; Baker and Cook, 1979; Meity , Sinaya and Quimio,1987). Aerobiological sampling methods are diversed based on different scientific principles and vary according to individual interests in the component of the aeromicrobiota (Tilak,1989).Research on aerobiological approaches to crop diseases are well established in many parts of India. However, for North-East India it has been a virgin field. Baruah is reversed as the father of Aerobiology in North Eastern India (Singh,2006). Many plant pathogenic fungi are known to produce spores in almost incal-

culable number , the result may be catastrophic when conditions are favourable for widespread dissemination, germination and infection on susceptible crop plant. The present paper deals with aerofungi over cabbage field in Moreh – a border town of India and Myanmar.

MATARIALS AND METHODS

Aeromycological studies

Aeromycological surveys over cabbage field were carried out with the help of two stage volumetric Andersen sampler (Andersen,USA), for two years (January 2012- December 2013). Culture plate method was also employed for identifying airborne fungal spores. The Petriplates (in duplicate) were exposed for ten minutes and then the exposed Petriplates were incubated in an inverted position for 3-5 days at 27ÚC ±1ÚC. The fungal colonies so developed were isolated, examined and identified using the standard literatures (Ellis,1993 ;Gilman,1956, Raper and Fennell, 1973)

Isolation and identification of causal organism of cabbage

The infected cabbage leaves were collected and the isolation of the organism was done in aseptic condition under Laminar Air flow chamber. The samples collected were surface sterilized using 0.01% mercuric chloride solution followed by washing in sterile distilled water. The infected pieces were inoculated into Petriplates containing PDA media. The Petriplates were then incubated at $27^0C \pm 1^0C$ and then identified with the help of published literatures.

Evaluation of the effect of phytoextracts on the growth of fungal pathogen

25 g each of fresh leaves of *Tajeta petula, Allium tuberosum, Melia azedarach, Justicia adatoda, Cassia tora, Centella asiatica, Vitex trifolia, Solanum anguivii, Mentha spicata* were taken. Plant parts were first washed thoroughly 2-3 times with tap water and then again with sterilized distilled water. The surface sterilization was done finally with 90% ethanol. The plant materials were crushed in 100 ml sterile distilled water. The maserate was filtered through double layer cheese cloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Whatman No.1 filter paper. Extract (75%), thus obtained was utilized for further investigations. The plant extracts was kept at 6ÚC till the tests were carried out. The aqueous extracts were sterilized at 121°C for 15 min prior to use.

Chemical fungicides against Gliocladium penicilloids

Study was made on the effects of three systemic fungicides *viz.*,(a) Carbendazim 50 % W.P.(b) Mancozeb 75 % W.P. and (c) Thiophanate Methyl 70 % W.P. on *Gliocladium penicilloids* causing leaf disease on cabbage *in vitro.* Fungal toxicity effect of the selected fungicides were tested against *G. penicilloids*. The fungicides were used with three concentrations, *viz.* 0.05 %, 0.075 % and 0.1% and added to sterilized PDA medium after mixing thoroughly and 20 ml of this mixture was transferred to each petriplate (9 cm diameter). Petriplate without fungicides was served as control. In each case, three (3) replicates were taken. The Petriplates were inoculated aseptically with mycilial colony bits (9 mm) removed from 7 day old pure culture of *G. penicilloids* and incubated at 26±1Ú C till the mycelial growth in the control reaches a maximum growth (120 h). The diameters of the colonies were measured after every 24 h and average values compared with control were taken as a measure of fungal toxicity. Growth inhibition (%) of test fungal was determined by using the following formula (Pani and Patra, 1997).

$$\text{Growth inhibition percentage (\%)} = \frac{\text{Control–Treatment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

A total of 37 fungal types were recorded by using the two stage volumetric Andersen sampler. The maximum fungal colonies were recorded in the month of November 2013 and minimum was recorded in the month of May 2012. *Aspergillus fumigatus* was found to be dominant type occupying the highest position (15.4%) followed by *Gliocladium penicilloids*(13.7%) and *Aspergillus nigers* (13.2%) (Table 1). The presence of *Aspergillus* species in higher concentration in South Assam was also reported by Sharma and Dutta. (2001).

Inhibitory effects of the selected phytoextracts on the radial growth of *Gliocladium penicilloids* were

Table 1 : Monthly mean data of airborns fungal spores found over the Cabbage filed in Moreh area (2012-2013)

Fungal type	January		February		March		April		May		June		July		August		September		October		November		December		Total	% Contribution
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013		
Aspergillus clavatus	-	-	1	-	-	-	4	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	-	8	0.7
A.fumigatus	29	9	5	12	15	24	27	11	6	14	7	12	9	2	2	11	13	19	9	18	24	32	7	10	170	15.4
A.humicola	-	-	1	-	-	-	3	-	1	-	-	1	-	-	-	-	-	-	2	-	-	-	-	1	8	0.72
A.nidulans	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	-	-	4	0.36
A.niger	7	3	3	9	9	12	7	2	10	12	5	2	5	1	8	2	2	9	11	4	6	12	4	1	146	13.2
A. flavus	1	5	5	13	9	5	3	11	-	6	3	1	1	4	2	1	19	7	8	5	2	7	3	6	128	11.61
A. ochraceus	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0.27
A. oryzae	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4	2	-	-	-	-	-	-	8	0.72
Alternaria alternata	-	-	2	-	-	-	2	5	-	-	-	-	3	-	-	1	-	2	-	-	7	-	-	-	22	1.99
A.peponicola	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3	0.27
Biospora sp.	-	-	-	2	-	-	-	-	-	1	-	-	-	-	1	-	-	-	6	2	-	-	-	-	12	1.09
Botrytis sp.	-	-	-	7	1	-	-	-	-	-	2	-	-	1	-	-	-	-	-	2	2	-	6	2	23	2.09
Candida sp.	6	2	-	-	-	-	-	-	-	-	1	-	-	-	2	1	-	-	-	2		5	5	1	25	2.27
Cladosporium cladosporoides	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	1	4	0.36
Curvularia lunata	-	-	-	-	6	-	2	1	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	2	15	1.36
Curvularia sp	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	4	2	1	-	-	-	9	0.82
Drechslera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	2	0.18
Epicocum nigrum	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	3	0.27

Fusarium oxysporum	-	1	-	-	-	5	-	2	-	-	-	-	5	-	1	-	1	-	-	1	-	2	-	-	18	1.63
Geotrichum sp.	-	-	1	--	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.18
Humicola sp.	-	-	-		-		4		-		-		2		-		5		1		-		-		12	1.11
Helmintho-sporium sp.	-	4	1	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	8	0.72
Mucor sp.	-	1	-	3	-	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-		-	-	7	0.64
Penicillium citrinum.	2	5	-	2	4	-	-	-	-	-	-	-	1	-	6	2	-	-	1	2	-	-	-	-	25	2.54
P. glanulatum	-	-	5	-	7	-	2	2	1	-	-	-	9	12	4	2	7	4	3	-	-	1	-	-	59	5.36
P. notatum	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.36
P.chrysogenum	3	1	1	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-	-	4	-	-			13	1.18
P.italicum	-	-	4	2	15	-	2	-	4	-	-	-	1	-	-	2	-	-	1	-	-	5	1	1	38	3.45
P. expensum	3	-	-		-	1	-	-	-		-	2	-	1	-	16	-	4	-	-	1	-	-	5	33	2.99
P. granulatum	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	5	-	1	3	-	7	-	4	-	23	2.09
P. spinulose	-	4	-	-	1	-	5	-	4	14	-	-	2	-	-	-	-	-	5	-	2	-	2	-	39	3.54
Rhizopus sp.	-	-	4	-	19	-	2	-	-	-	-	-	-	-	7	-	-	-	-	1	2	-	-	-	35	3.18
Smut spores	6	2	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2	5	18	1.6
Torula sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	3	0.27
Trichoderma harzianum	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	4	0.36
T.viridae	-	-	3		7		-		-		-		-		-		-		-		1		5		16	1.45
Gliocladium penicilloids	2	4	4	6	1	3	12	5	9	4	12	1	3	14	-	2	4	13	8	10	7	13	1	2	152	13.7
Grand total																									**1102**	99.69

Table 2 : *In vitro* evaluation on effect of nine selected phytoextracts on the linear mycelium growth of *Gliocladium penicilloids*

Phytoextract	Control (mm)	5%	10%	15%	20%
Tajeta petula	43.2	39.3 ± 0.27	37±0.98	33±1.65	30±1.69
Allium tuberosum	43.2	34 ± 2.16	32.3±2.12	28±1.90	27±2.16
Melia azedarach	43.2	30.3 ± 1.90	28.6±2.12	27±2.94	26.3±1.90
Justicia adatoda	43.2	31 ± 2.86	30±2.16	29.6±4.01	27±4.02
Cassia tora	43.2	42.3 ± 1.44	38.6±3.07	36±3.091	34±4.49
Centella asiatica	43.2	37 ±0.94	35±1.96	34±2.94	32.6±6.50
Vitex trifolia	43.2	40 ±2.16	37±0.98	32.6±2.12	31±5.50
Solanum anguivii	43.2	34 ±3.68	30.3±1.52	29.3±3.14	25±1.91
Mentha spicata	43.2	40 ±3.56	37±3.09	35±3.31	33±2.12
SEm		3.20	2.45	4.47	6.26
CD (0.05)		6.78	5.19	9.47	13.27

Table 3 : *In vitro* evaluation on the effect of three systemic fungicides on linear mycelium growth of *Gliocladium penicilloids*

Fungicide (120 hrs)	Control	0.05%	0.075%	0.1%
Thiophanate methyl	43.2	7.3±0.72	7.67±1.89	7±0
Carbendazim	43.2	8.33±0.27	8±2.03	7.33±0.72
Mancozeb	43.2	15±2.83	14±1.41	9.67±1.44
SEm		3.03	1.55	1.75
CD 0.05		8.43	4.31	4.87

found to be different. Minor inhibitory effect was found in all the concentrations by nine phytoextracts against *Gliocladium penicilloids.* Out of the nine selected phytoextracts, *Solanum anguivii* (21.3%,29.9%,32.2% & 42.1% at 5%,10%,15% & 20%), *Melia azedarach* were found to have highest inhibitory effect (29.9%,33.7%,37.5%&39.1% at 5%,10%, 15% & 20%) followed *Allium tuberrosum* (21.3%, 25.2%, 35.2% & 37.5% at 5%, 10%, 15% & 20%) respectively (Table 2). All the fungicides inhibited the radial growth of the fungi in the culture of PDA medium amended with different concentration of fungicides, but showed variations in the extend of inhibition. All the chemical fungicides were more inhibitory with increase in concentration. Out of the three fungicides selected, Thiophanate methyl suppressed the growth of *Gliocladium penicilloids* only at 0.05%, Similarly, in case of Carbendazim, higher inhibitory effect was found in lower concentration i.e., 83.1% at 0.05% whereas, Mancozeb showed less inhibitory effect in lower concentration i.e., 65.3% at 0.05% and also was less suppressive in higher concentration comparing to Thiophanate methyl and Carbendazim (Table 3). Thiophanate methyl treated plants gave a better yield than the non-infected control plants had also been reported by Dutta (1980).

The present study revealed the application of fungicides was more effective than the application of phytoextracts. However, the disease controlled by biocontrol agent reduced problems such as damage to bio-diversity, toxic to non- target species, etc. created by the application of fungicides (Romesh, 2012). Application of streptocycline after pruning or sprying in combination with copper oxycloride was found to be effective in reducing the citrus bacterial canker pathogen on acid lime (*Citrus aurantifolia*) under field condition in gangetic plains of West Bengal (Hansda *et al.* 2013). Among the chemicals Mancozeb is the best one for managing Early Blight disease of Tomato as well as from the fruit yield point of view (Saha *et al.* 2013). The next two were Difenconazole and Hexaconazole but in our study mancozeb was found to be less effective to *Gliocladium penicilloids*

it might be due to less susceptibility of the organism to mancozeb. The most damaging diseases of cabbage were not controlled easily with fungicides or bacteriocides. Hence, we may rely on crop rotation, clean cultural practices, resistant varieties and water management to lesser diseases. Moreover, aeromycological studies have to be taken into consideration for disease forecasting system of air borne pathogen. *Gliocladium penicilloids* isolated from cabbage grown in Moreh area of Manipur will be a new addition to fungal diseases of cabbage.

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