

Original Research Article

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## Morphology and *invitro* Study of Chemicals, Plant Extracts, Bio-Agents against *Pestalotiopsis mangiferae*, Causing Grey Leaf Spot of Mango in Manipur, India

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### ABSTRACT

#### Keywords

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A study was conducted *in vitro* condition to analyse the morphology and efficacy of bio agent, plant extract and some chemical against *P. mangiferae* that were collected from different districts of Manipur. The morphological character under study consists of colony and conidial characteristics such as colour, shape, size and appendages. The cultural growth colour of *P. mangiferae* on PDA differed from concolour to versicolour fuliginous. The conidial shape varied from oval and spherical to elliptical with prominent appendages. The conidial length and width were 22.9 and 5.7  $\mu\text{m}$  respectively. The diseased sample which was collected from different district of Manipur consists of three septation and the number of conidial appendages was found 2-3 numbers. Among seven antagonists namely *Penicillium citrinum*, *Trichoderma atroviride*, *T. ovalisporum*, *Hypocrealixii*, *T. harzianum* (69 & 131) and *T. asperellum* evaluated *in vitro*, *T. asperellum* revealed the best in inhibiting the growth of the fungus (85.8%). Among three plant extracts *viz.* garlic, neem and sweet flag evaluated *in vitro*, garlic extract (3.0%) showed the best result (100.0%). Among the seven chemicals *viz.* carbendazim, thiophenate methyl, mancozeb, imidacloprid, fipronil, profenophos and thiomethoxam evaluated *in vitro*, thiophenate methyl (0.05%) and carbendazim (0.05%) showed the best result with 100.0 per cent inhibition in fungal growth.

### Introduction

*Mangifera indica* (Anacardiaceae) is one of the important fruit tree which is grown commercially both in tropical and subtropical region. Mango is known as king of fruits and it's a National fruit of India. The genus *Mangifera* originated in South-East Asia at an early date. Records suggest that it has been in cultivation in the Indian subcontinent for well

over 4000 years now (De Candolle, 1904) and has been, since time immemorial, one of the most favourite of the kings and common people among fruits because of its delicious taste and fascinated flavour. According to Mukherjee (1958), the natural spread of genus is limited to the Indo-Malaysian region, extending from India to the Philippines and New Guinea in the east. India is the largest producer and contributing 40.75% of the total

fruit production with 19.69 million tonnes production and 8.7 million tonnes/ha productivity (NHB, 2017). In India, the important states of mango producing viz. Andhra Pradesh, Bihar, Gujarat, Maharashtra, Odisha, Tamil Nadu, Karnataka, Uttar Pradesh and West Bengal. Apart from India, the other major producers of mango in the world are Brazil, Pakistan, Mexico, Indonesia, the Philippines, Haiti, China and Tanzania. Mango is also grown to a small extent in Egypt, Madagascar, Zaire, Venezuela and Sri Lanka. The climate condition of Manipur is well suited for growing of Mango.

Young and unripe mango, due to their acidic taste, are used for culinary purposes as well as for preparing pickles, chutneys and amchoor. Ripe fruits are used in preparing of squash, nectar, jam, cereal flakes, custard powder and baby food, mango leather (am-paper) and toffee. Moreover, fruits of some cultivars like Alphonso and Dashehari are sliced and canned for catering during the off-season.

Alphonso was found to be the most suitable for canned juice preparation due to its very good colour, Consistency and flavor, followed by Padiri (Gowda and Ramanjaneya, 1995). The finest cultivars for processing were Haden, Haden 2H, Glenn, Joe Welch and Torbet, which had very good organoleptic characteristics (Donadio, 1995). High carotenoid content in Amrapali gives it potential for juice production and blending (Khurdiya and Roy, 1989).

The limiting factor in fruit yield is due to the occurrence of diseases. The pathogen (*P.mangifera*) causes infection on leaf, twigs and panicle. As a result the vegetative growth and the fruit production of young mango plants were reduced. In Manipur, grey spot of mango was first observed by Iboton & Tombisana (2012) and little work on this disease was done by Indira (2014).

## Materials and Methods

Surveys were conducted during September to November, 2017 on grey leaf spot of mango at different locations in the valley districts of Manipur namely Imphal East, Imphal West, Thoubal and Bishnupur (Plate 2). The diseased samples were brought to laboratory for isolation. The diseased samples were cut into small pieces of 2-3mm size.

These pieces were surface sterilized with 1% sodium hypochlorite solution for 2-3 minutes. The sterilized pieces were then inoculated on PDA Petri dish. The inoculated Petri plates were incubated at  $28 \pm 1^{\circ}\text{C}$  for 4 days. Daily observations were taken on the development of the pathogens. The fungal culture was purified by adopting hyphal tip cut method from the actively growing hyphal tips.

The pure culture was maintained on freshly prepared Potato Dextrose Agar slants inside the refrigerator and periodically sub cultured to fresh medium during the experimental period. Pathogenicity test was conducted by the method described by Koch (1876).

Per cent inhibition on growth was calculated which was described by Vincent (1927). The antagonistic activities was recorded by following dual culture technique using modified Bell's scale described by Bell *et al.*, (1982) using PDA as basal medium.

## Results and Discussion

The grey leaf spot of mango was found at various mango growing locations in different valley districts of Manipur (Table 1). Survey results indicated that there is prevalence of grey leaf spot of mango (plate 1) in Manipur, Rattan (2009) reported that grey blight of mango caused by *P.mangiferae* (P. Henn.) Stey is prevalent in many states in India.

## Morphological study

*P.mangiferae* was consistently isolated from the diseased samples of grey leaf spot of mango. The present findings is in agreement with those of Ko *et al.*, (2007) who reported that single conidial isolates of the fungus were identified morphologically as *P. mangiferae* and were consistently isolated from the diseased mango leaves on PDA medium. Similarly, Keith and Zee (2010) who reported that *Pestalotiopsis*, *Colletotrichum*, *Mucor* and *Guignardia* were the agents of leaf spot of guava in Hawaii (Table 2 and Plate 2).

The conidial size of *P. mangiferae* ranged from 22.4-24.8  $\mu\text{m}$  in length and 5.8-6.7  $\mu\text{m}$  in width. The numbers of conidial appendages were found to be 2-3 in number. Cultural growth colour of *P. mangiferae* on PDA varied from concolour to versicolour fuliginous with varied shape such as oval and spherical to elliptical with prominent appendages. There was three septation in all the conidia collected from different places. Germ tube of the conidia at 8 hours ranged from 5.5-7.0  $\mu\text{m}$ , at 16 hours, 41.0-43.0  $\mu\text{m}$  and at 24 hours, 47.0-49.0  $\mu\text{m}$  during incubation. The present finding is in agreement with the finding of S.K Singh (2011) who reported that *Pestalotiopsis* isolates were recorded with minor variations and homogenous conidial lengths (22.2 to 27.1  $\mu\text{m}$ ), conidial width (5.5 to 6.9 $\mu\text{m}$ ) and size of appendages (4.0 to 6.7  $\mu\text{m}$ ). However, three types of median colour cells were recorded among the isolates. Ranjana das *et al.*, (2010) reported that the germination of spores started at 8 hours of incubation and gradually increased upto 20 hours (maximum).

## Pathogenicity test

The fungus isolated from the diseased samples was found pathogenic when

artificially inoculated on healthy excised leaves of mango could induced disease symptom after 7 days of incubation. On reisolation the same pathogen was found and thus proved pathogenicity of the pathogen (plate 3 & 4). The present findings is in agreement with the findings of Ko *et al.*, (2007) who reported that upon conducting pathogenicity tests, the symptoms of grey leaf spot were observed on all inoculated leaves, while uninoculated leaves remained completely free from symptoms. Reisolation from the inoculated leaves consistently yielded *P. mangiferae*.

## Efficacy of chemicals

The linear growth of *P.mangiferae* was completely inhibited by carbendazim (0.1 & 0.05%), thiophanate methyl (0.1 & 0.05%), and mancozeb at 0.1%. However, mancozeb (0.05%), Imidacloprid (0.1%), Fipronil (0.2%), Profenophos (0.1%) and Thiomethoxam (0.1 & 0.05%) showed 61.53, 35.23, 11.70, 88.33 and (43.43 & 17.57) % inhibition over control respectively. Fipronil (0.2%) showed the least inhibition of 11.70% on linear growth of the pathogen. The fungus failed to sporurate in all the fungicides tested. The inhibitory effect of thiophanate methyl and mancozeb on growth of fungus might be due to inhibition of nucleic acid synthesis, melanin synthesis and disrupting lipid metabolism while inhibitory effect of carbendazim might be due to disruption of enzyme synthesis, interfere with nuclear division of fungus. The present findings are in agreement with those of Kudalkar *et al.*, (1991) who reported that growth and sporulation of *P. mangiferae* was completely inhibited *in vitro* by carbendazim (100-300ppm). Mancozeb also inhibited growth by 78-86%. Similar findings were also reported by Selvan *et al.*, (1993) that *Pestalotiopsis palmarum* was completely inhibited under *in vitro* by Carbendazim and Thiophanate

methyl at all concentrations tested. And also according to Rahman *et al.*, (2013) who evaluated the efficacy of some selected contact, systemic and mixed fungicides against *Pestalotia palmarum* causal organism of grey spot of coconut (Table 3 and Plate 5).

**Antifungal properties of aqueous plant extracts**

Three plant extracts tested against mycelial growth of *P. mangiferae*, garlic extract (3 & 1.5%), neem extract (10 & 5%) and sweet flag extract (10 & 5%) could inhibit (100 & 77.76%), (82.35 & 67.05%) and (83.52 & 61.17%) respectively. The effectiveness of garlic is might be due to antifungal activity of Diallyl-disulphide, Diallyl-trisulphide and

ajoene (Table 4). The aqueous plant extracts under *in vitro* revealed that higher doses were relatively more efficient than the lower doses. The present findings are in agreement with the findings of Islam *et al.*, (2004) who reported that among the plant extracts tested *in vitro*, garlic @3% was most effective in inhibiting 100% radial growth of *P. mangiferae* (Plate 6).

Similarly, Saha *et al.*, (2005) reported the antifungal properties of ethanol and aqueous extracts of *Allium sativum* (L.), *Daturametel* (L.), *Dryopterisfilix-mas* (L.) Schott, *Zingiber officinale* (Roscoe.), *Smilax zeylanica* (L.), *Azadirachtaindica*, (A.) joss. And *Curcuma longa* (L.) on *Pestalotiopsis theae* (Saw.) and shown 100% inhibition of spore germination.

**Table.1** Survey of grey leaf spot of mango in Manipur

SL.No	Place of Survey		GPS: LOCATION		
	District	Site/Place	Latitude	Longitude	Altitude (ft.)
1	Imphal East	Andro KVK	24°45.885	094°03.274	2649
2	Imphal East	Thayong	24°47.828	094°04.583	2666
3	Imphal East	AndroMaringthel	24°44.289	094°01.097	3335
4	Bishnupur	Nambol	24°43.190	094°50.153	2537
5	Bishnupur	Ningthou-khong bazaar	24°34.249	094°45.829	2510
6	Bishnupur	Potsangbam	24°35.809	094°45.940	2560
7	Bishnupur	Utlou	24°43.286	094°51.538	2538
8	Thoubal	Waithou	24°40.326	094°57.452	2526
9	Thoubal	Kakchingsumangleikai	24°29.791	094°00.085	2507
10	Thoubal	Wabagaimakhaleikai	24°31.381	094°56.107	2498
11	Thoubal	Sekmaijingmakhaleikai	24°33.256	094°54.012	2514
12	Imphal West	Mayang Imphal thanaawangleikai	24°37.683	094°53.532	2489
13	Imphal West	Hiyangthang mamang leikai	24°43.698	094°54.372	2518
14	Imphal West	Iroisemba	24°45.161	094°02.606	2746
15	Imphal West	ICAR Lamphelpat	24°49.651	094°55.603	2538

**Table.2** Morphological study of different isolates of *P. mangiferae*

Isolate of <i>P. mangiferae</i>	*Conidial length(µm)	*Conidial width(µm)	Number of apical appendages (ranges)	Type of median colour	Shape	with	Septation
<b>1. AndroKvk</b>	22.8±1.0	6.8±0.9	2-3	Concolour	Oval prominent appendages	with	3
<b>2. Thayong</b>	24.8±0.6	6.3±0.9	2-3	Versicolour Fuliginous	Spherical prominent appendages	with	3
<b>3. AndroMaringthel</b>	23.5±1.0	6.2±0.7	2-3	Concolour	Oval prominent appendages	with	3
<b>4. Nambol</b>	24.9±0.8	6.6±0.7	2-3	Versicolour Fuliginous	Elliptical prominent appendages	with	3
<b>5. Ningthou-khong bazaar</b>	22.9±1.0	6.3±0.9	2-3	Concolour	Spherical prominent appendages	with	3
<b>6. Potsangbam</b>	24.8±0.8	6.3±0.9	2-3	Concolour	Oval prominent appendages	with	3
<b>7. Utlou</b>	23.4±1.0	6.2±0.7	2-3	Versicolour Fuliginous	Elliptical prominent appendages	with	3
<b>8. Waithou</b>	24.9±0.7	6.3±0.8	2-3	Versicolour Fuliginous	Oval prominent appendages	with	3
<b>9. Kakchingsu mangleikai</b>	22.4±1.0	5.7±0.9	2-3	Versicolour Fuliginous	Spherical prominent appendages	with	3
<b>10. Wabagaima khaleikai</b>	23.3±1.0	6.2±0.4	2-3	Versicolour Fuliginous	Oval prominent appendages	with	3
<b>11. Sekmaijingmakhaleikai</b>	22.8±1.0	6.1±0.2	2-3	Versicolour Fuliginous	Elliptical prominent appendages	with	3
<b>12. Mayang Imphal thanaawangleikai</b>	23.9±0.8	6.2±0.8	2-3	Concolour	Oval prominent appendages	with	3
<b>13. Hiyangthang mamang leikai</b>	22.9±0.5	5.4±0.8	2-3	Concolour	Elliptical prominent appendages	with	3
<b>14. Iroisemba</b>	24.2±0.2	6.4±0.3	2-3	Versicolour Fuliginous	Oval prominent appendages	with	3
<b>15. ICAR. Lamphelpat</b>	23.9±0.8	6.2±0.5	2-3	Concolour	Spherical prominent appendages	with	3

\*Mean of three replications

**Table.3** *In vitro* efficacy of chemicals on growth of *P. mangiferae*

Sl. No.	Treatment	Concentration	Inhibition (%) over control
1	Carbendazim	0.05%	*100 **(89.50)
		0.1%	100 (89.50)
2	Thiophenate methyl	0.05%	100 (89.50)
		0.1%	100 (89.50)
3	Mancozeb	0.05%	100 (89.50)
		0.1%	15.29 (51.99)
4	Thiomethoxam	0.05%	17.56 (36.36)
		0.1%	43.43 (20.03)
5	Imidacloprid	0.1%	35.23 (70.38)
6	Fipronil	0.2%	11.70 (41.27)
7	Profenophos	0.1%	88.33 (46.67)
	SE (d) ±		3.87
	CD <sub>(0.05)</sub>		6.36

\*Mean of three replication

\*\*Figures in parenthesis are arcsine transformed value

**Table.4** Effect of plant extracts on the growth of *P. mangiferae in vitro*

Treatment	Plant part used	Concentration %	Inhibition % over control
Garlic	Clove	1.5	*71.76 **(89.32)
		3	100.00 (57.90)
Neem	Leaf	5	67.05 (65.16)
		10	82.35 (56.30)
Sweet flag	Rhizome	5	61.17 (66.06)
		10	83.52 (51.45)
SE(d)			0.72
CD <sub>(0.05)</sub>			1.59

\* = Mean of three replications

\*\*Figures in parenthesis are arcsine transformed value



**Table.5** Effect of bio control agents on the growth of *P. mangiferae* in vitro

SL. No.	Biocontrol agent	Duration point of contact(days)	Bell's scale	Inhibition over control (%)
1	<i>Penicillium citrinum</i>	-	Class iv	*47.52 **(43.53)
2	<i>Trichoderma harzianum</i> (KU933468)	2	Class i	63.17 (52.62)
3	<i>T. harzianum</i> (KU933474)	2	Class i	64.00 (53.08)
4	<i>T. ovalisporum</i> (KU904456)	3	Class vi	74.58 (59.68)
5	<i>T. hypocrea</i> (KX0113223)	3	Class vi	60.47 (49.07)
6	<i>T. asperellum</i> (KU933475)	3	Class i	85.88 (67.93)
7	<i>T. atroviride</i> (KU933472)	2	Class i	84.00 (66.35)
<b>SE(d)</b>				2.57
<b>CD<sub>(0.05)</sub></b>				5.53

\*Mean of three replications

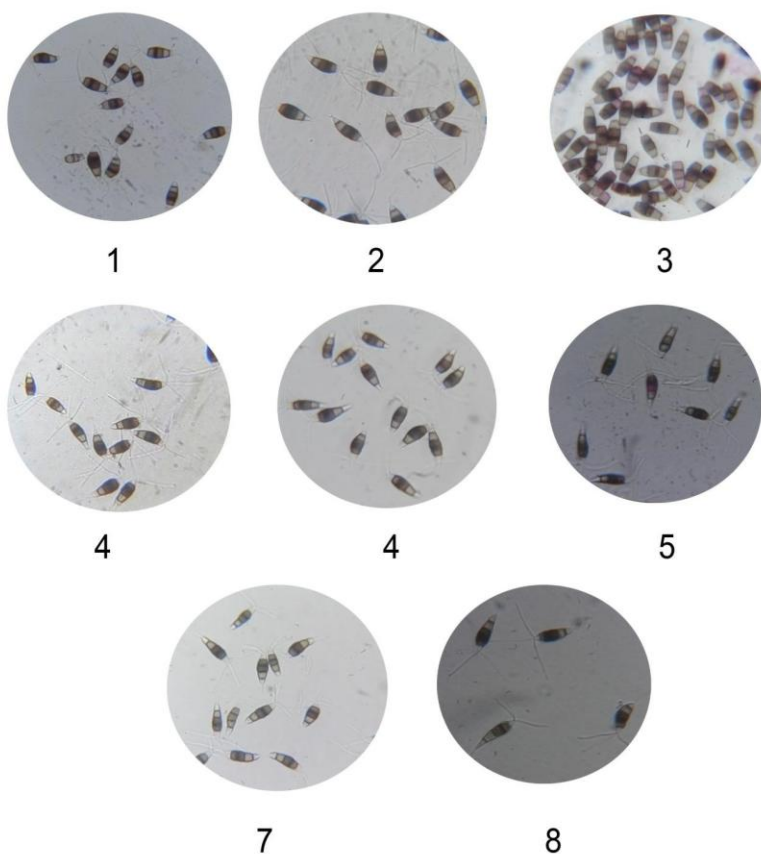
\*\*Figures in parenthesis are arcsine transformed value



**Plate.1** Symptoms of grey leaf spot of mango from different areas of Manipur A=Imphal West, B=Imphal East, C & D=Thoubal and E & F=Bishnupur

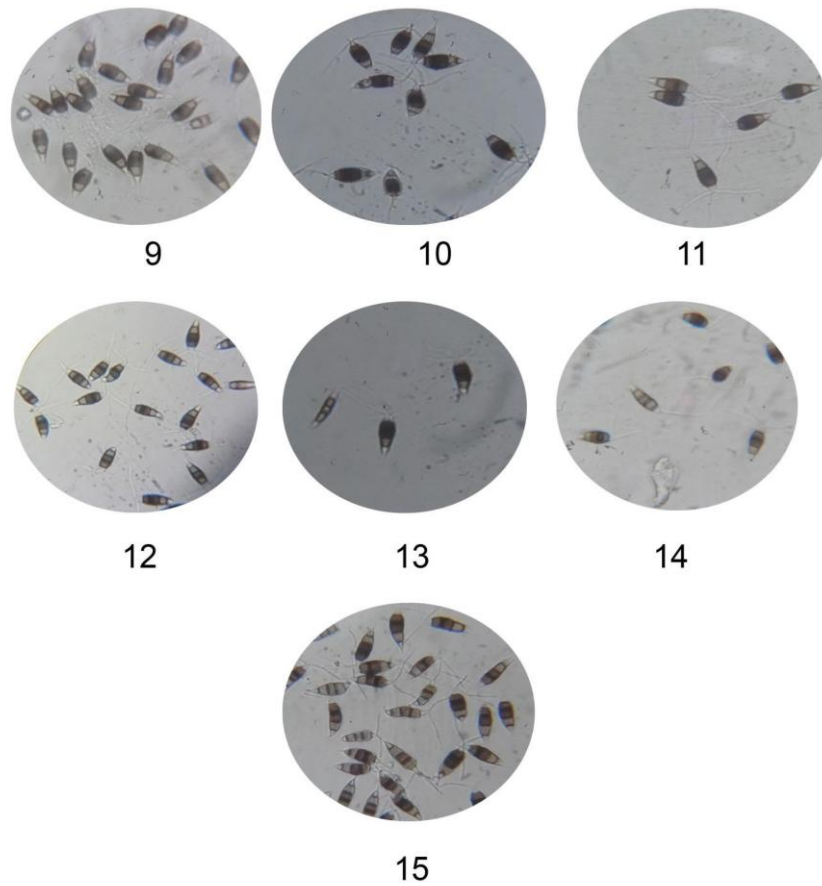


**Plate.2** Different isolates of *P. mangiferae* (1=Andro KVK, 2=Thayong, 3=AndroMaringthel, 4=Nambol, 5=Ningthoukhong, 6=Potsangbam, 7=Utlou, 8=Waithou, 9=Kakching, 10=Wabagai, 11=Sekmajing, 12=Mayang Imphal, 13=Hiyangthang, 14=Iroisemba, 15=ICAR Lamphelpat)

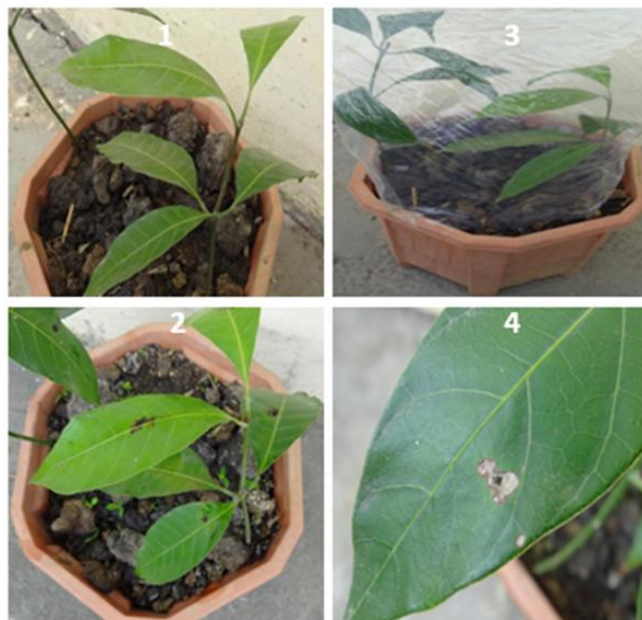


**Plate.3(A)** Conidia of different isolates of *P. mangiferae* (1=Andro KVK, 2=Thayong, 3=AndroMaringthel, 4=Nambol, 5=Ninthoukhong, 6=Potsangbam, 7=Utlou, 8=Waithou)

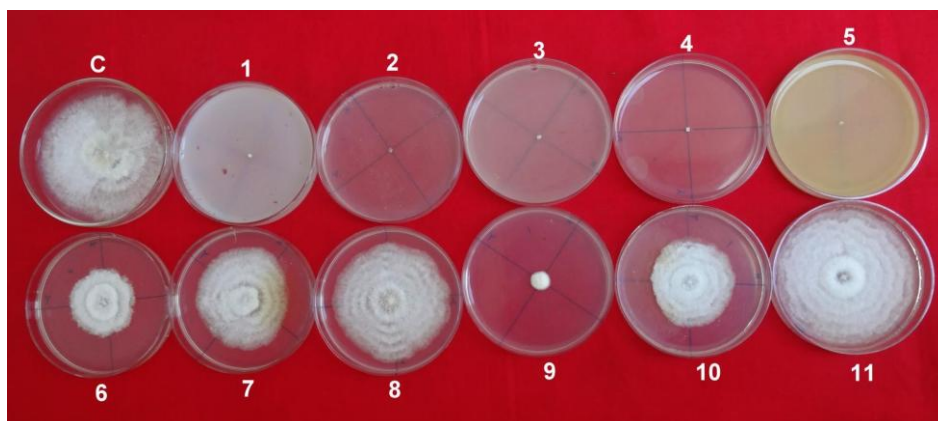




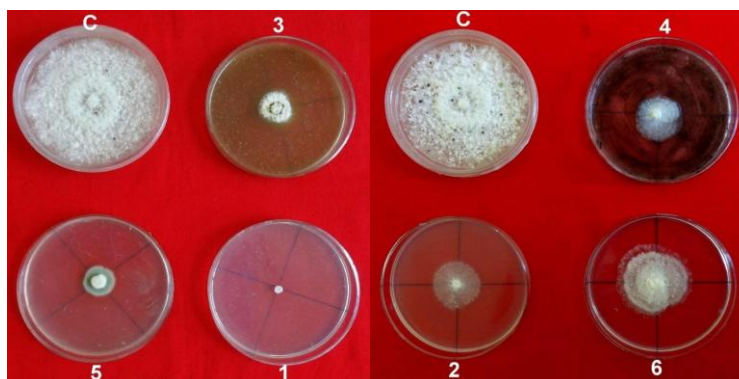
**Plate.3(B)** Conidia of different isolates of *P. mangiferae*(9=Kakching 10=Wabagai, 11=Sekmaijing, 12=Mayang Imphal, 13=Hiyangthang, 14=Iroisemba, 15=ICAR Lamphelpat)



**Plate.4** Pathogenicity test of *P. mangiferae* (Stepwise, 1-4)



**Plate.5** *In vitro* efficacy of chemicals on growth of *P. mangiferae*(C=Control, 1=Carbendazim 0.1%, 2=Carbendazim 0.05%, 3=Thiophenate methyl 0.1%, 4=Thiophenate methyl 0.05%, 5=Mancozeb 0.1%, 6=Mancozeb 0.05%, 7=Imidacloprid 0.1%, 8=Fipronil 0.2%, 9=Profenophos 0.1%, 10=Thiomethoxam 0.1%, 11=Thiomethoxam 0.05)



**Plate.6** Effect of plant extracts on the growth of *P. mangiferae* [C=Control, 1=Garlic (3%), 2=Garlic (1.5%), 3=Neem (10%), 4=Neem (5%), 5=Sweet flag (10%), 6=Sweet flag (5%)]



**Plate.7** Effect of bio control agents on the growth of *P. mangiferae* (C=Control 1=*Penicillium citrinum*, 2=*Trichoderma harzianum* (KU933468), 3=*T. harzianum* (KU933474), 4=*T. ovalisporum*, 5=*T. hypocrea*, 6=*T. asperellum*, 7=*T. atroviride*)

### Effect of biocontrol agents

Findings revealed that three species of *Trichoderma* could come in contact with the pathogenic fungus after 2 days of incubation. *P. mangiferae* was overgrown by 100 % (Class I) by the antagonists *T. harzianum* 69, *T.harzianum* 131, *T. asperellum*7 and *T.atroviride*. *Penicillium citrinum* remained locked with the fungus at the point of contact (Class IV). However, the antagonists *T. ovalisporum* and *T. hypocrea* could not overgrow the fungus but formed inhibition zone of 2.16cm and 3.36cm (ClassVI). The present findings are in agreement with those of Bhuvaneswari and Rao (2001) who reported that *T. viride* could inhibit 72.88% on the growth of *Pestalotia* sp. Similarly, Bhargava *et al.*, (2003) also reported that *T.harzianum* could inhibit the mycelial growth of *P.dessiminata*. The antagonistic effects of species of *Trichoderma* against pathogen might be due to (a) production of non-volatile toxic substances diffuse in the substrate and (b) parasitism of the antagonists. The formation of inhibition zone indicates the production of antibiotics by the antagonists which diffused in the medium (Table 5 and Plate 7).

The fungus causing grey leaf spot of mango was well distributed in all localities in different districts of Manipur. The severity of disease was found highest from late September to November, 2017. Among all the treatments, fungicides (thiophanate methyl and carbendazim) were most effective which was closely followed by plant extract (garlic extract) and bio control agent (*T. harzianum*). Even though fungicides were very effective, they are considered harmful to human health and detrimental effect on environment.

As a substitute of fungicides, effective plant extracts or bio control agents could be used as one of components of integrated disease

management. The increased in protein synthesis in plant attacked by pathogen reflects the increased production of enzymes and other protein involved in defence reactions of the plant. Therefore, timely application of management practices will increased the resistant capacity of the plant which will be directly proportional to plant yield. These findings will be greatly helpful to the mango farming community in Manipur. Hence, further research work on the epidemiology, its survival and spread may be taken up for reducing the incidence of disease.

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