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RESULTS

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### Isolation

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Forty two fungi including *F.moniliforme* and a sterile mycelium were isolated from leaves, flowers, roots, stems and fruits of all varieties of *M. indica* studied (Table 1). Alternaria alternata, Botryodiplodia theobromae, *Curvularia hinata, Chaetomium globosum, Cephalosporium* sp., *Cladosporium oxysporum, Drechslera specifer, Fusarium solani, Fusarium pallidoroseum, Gloeosporium mangiferae, Humicola grisea, Pestaloĝiopsis* $l_{i}^{9}$ *) mangiferae,* and *Thielaviopsis paradoxa* were isolated frequently from leaves (Fig. 2A-G).

The intensity of mango malformation disease (Fig.1C) was found to be more prevalent in the variety *rajapuri* as compared to the other varieties. The fungus *F. moniliforme* (Fig. 2H) was commonly found associated with every part of the malformed flowers (Fig. 3A, B) and with vegetative malformed shoot (Fig. 3C, D). Pathogenicity test was also revealed positive response with *F. moniliforme* (Fig. 3E).

Mango fruits, particularly the ripened ones were more suceptible to fungal disease. *Aspergillus* rot, *Botryodiplodia* rot (Fig. 1A), Anthracnose, *Phoma* rot were the common diseases associated with ripened mango fruits.

The secondary Xylem (wood) of main trunk particularly the discolored portion showed the fungal association. Aspegillus ellipticus, Aspergillus niger, Cephalosporium sp., Chaetomium sp., Botryodiplodia theobromae (Fig. 2 I), Fusarium moniliforme, Pestalotiopsis versicolor and Theilaviopsis paradoxa were found associated with the wood elements.

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Aspergillus nidulans var. nidulans, Fusarium solani, Paecilomyces sp. and Trichoderma sp. were isolated frequently from roots. However, Fusarium moniliforme was not found in association with the root tissue.

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 Table 1 : List of fungi isolated from different parts of different varieties of

 M. indica.

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	Organisms	L	Fl o.	Fr.	St.	R
1 A	lternaria alternata (Fr ) Keissler	+	+	+	-	-
2 A	spergillus ellipticus	-	-		+	7
3 A	spergillus nidulans var. latus Thom & Raper.	+	-		-	-
4 A	spergillus àidulans var. nidulans		-			+
5 A	spergillus niger Van Tiegh	-	+	+	+	-
6 B	otryodiplodia theobromae Pat	-	+	+	+	-
7 (.	'ephalosporium'[Corda.		-		+	-
8 (	Chaetomium globosum Kunze ex Steud.	+	-	+	-	-
9 C	Chaetomium sp.	+	+	+	+	-
10 C	Cladosporium cladosporioides (Fres.) de varies	+		-		-
11 (	Ladosportum oxysporum Berk & Curt	+			-	-
12 (	Colletotrichum sp	+		-	-	-
13 (	urvularia lunata (Wakker) Boedijn	+	-	-	_	-
14 (	'urvularia lunata var. aerta (Batista & Lina	+	+	-	-	-
1	aconcetos)					
15 L	Drechslera hawauensis (Bugni Court)	+	-			-
16 I	Drechslera specifer (Bain) Nicot.	+	-			-
17 L	emericella Berk	+	***	-		-
18 F	Fusarium garminearum			+	-	
19 <i>I</i>	Fusarium moniliforme Scheldon.	-	+		+	
20 /	usarium oxysporum Schlecht			+		
21	Fusarium pallidoroseum	+	-	_	-	
22	Fusarium solani (Mart.)	+		-		
23 (	Gloeosporium mangiferae (P. Henn) Stey.	÷		+	-	
24 (	Gonatobotrys simplex	+	+	_	_	
25	Helicosporium sp	-		_	+	
26	Helminthosporium sp.	+				
27	Humicola grisea Tragen	+				
28	Macrophomina phaseolina	+				
29	Monila sp	+	+	+		
30	Nigrospora sp.	+	+	÷-	_	
31	Paecilomyces lilacinus	_	-	_		
32	Pestalotiopsis mangiferae P. Henn.	÷	+	+	+	
33	Pestalotionsis versicolor (Spcg.)	+	- +		+	
34	Phomopsis amarali (Srirastava et al)			+		
35	Phomopsis sp.	+	+	_		
36	Rhizopus sp.		+	+		
37	Sclerotium rolfsii Sac.	+		+		
38	Thielavia terricola (Gilman & Abbott) Emmons	+		_		
39	Thielaviopsis paradoxa (de Sevnes) Hoenn.	+	. +		+	
40	Trichoderma harzianum Ritai	+	+	-		
41	Trichoderma viride Pcss	+	+	+		
42	Sterile mycelium	+	+	+	+	

L. - Leaves, Flo. - Flowers, Fr - Fruits, St.- Stem, R.- Root

Fig 1 : Disease symptoms caused by fungi on fruit (A), leaf (B) and inflorescence (C)

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A - Healthy (H) and diseased (I) fruit of variety rajapuri

- B Healthy (H) and infected (1) leaves of variety rajapuri
- C Healthy (H) and malformed (I) panicle of variety rajapuri



FIG.1

Fig 2 · A - 1 Different types of fungi isolated from different plant organs.

A-Drechslera specifer from leaf  $\times$  500

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B- Alternaria alternata from leaf × 250

C- Curvularia lunata from leaf  $\times$  1000

D- Gloeosporium mangiferae from leaf × 500

E- Thielaviopsis paradoxa from leaf  $\times$  500

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F- Pestalotiopsis mangiferae from leaf × 500

G- C'haetomum globosum from leaf  $\times$  200

H- *Eusarium moniliforme* from malformed panicle × 500

I- *Botryodiplodia theobromae* from wood × 500



FIG 2

- Fig 3 Association of *Fusarum moniliforme* with floral (A, B, E) and vegetative (C, D) organs
  - A Association of fungus with different organs of malformed flowers.
  - B A circular mat of *Fusarium monliforme* growing around the anther from malformed flower. Note the anther from healthy flower (Arrow) showing no growth of *Fusarium*.
  - C & D Fusarium growing from cut ends of axillary buds (C) and shoot buds (D) of malformed shoot.
  - E Pathogenicity test on flowers conforming the association of *I*: monultforme with malformed flowers Arrow indicates control flower



FIG.3

#### **PHYTOCHEMICAL CHANGES**

#### Post infectional phytochemical changes in healthy, infected and

#### fungus treated leaves of different varieties of *M. indica* L.

var. *rajapuri*. The distribution of tannins, saponins, iridoids, proanthocyanidins, alkaloids, flavonoids, mangiferin and phenolic acids in the healthy, infected and treated leaves is presented in the Table 2.

The healthy, infected and *Alternaria alternata* treated leaves of var. *rajapuri* contained saponins, alkaloids, flavonoids like quercetin and quercetagetin, phenolic acids like vanillic acid, syringic acid, p-OH benzoic acid and gallic acid and mangiferin. Apart from these 3'OMe quercetin was found in infected and fungus treated leaves The concentration of phenolic acids was found to be higher in infected and treated leaves. Infected and treated leaves also contained higher concentration of mangiferin. (Table 9).

Bioassay test was carried out against *Alternaria alternata* with plant leachate at different concentrations (2, 4, 6, 8 and 10 ml). The mycelial growth inhibition (82 4%) was maximal at 10 ml (Fig. 5A). Spore germination inhibition was also checked in different concentrations of plant leachate. At 10 ml, 85% of spore germination was inhibited (Fig. 9A).

var. *totapuri*: The distribution of phytochemicals in healthy, infected and fungus treated leaves of var. *totapuri* is presented in Table 3.

The healthy, diseased and fungus treated leaves contained saponins, alkaloids, mangiferin, phenolic acids like vanillic acid, syringic acid, p-OH benzoic acid and gallic acid. Apart from these healthy leaves also contained quercetin. Concentration of mangiferin (Table 9), p-OH benzoic acid and gallic acid was found to be very high in diseased and treated leaves.

Bioassay test with mangiferin against *Curvularia lunata* showed that the colony growth of mycelia was restricted at the maximum concentration. At 100 ppm concentration of mangiferin about 75% of colony growth was inhibited (Fig 4A). Spore germination of *Curvularia* was also carried out in different concentrations of mangiferin. The inhibition of spore germination was maximum (87%) at 100 ppm concentration (Fig 8B).

var. *ladva*: The distrubution of phytochemical compounds in healthy, diseased and fungus treated leaves of *M.indica* L. var. *ladva* is presented in Table 4.

The healthy, diseased and *Drechslera specifer* treated leaves contained saponins, alkaloids, flavonoids like quercetin and quercetagetin, mangiferin, vanillic acid, syringic acid, p-OH benzoic acid and gallic acid. Concentration of quercetin, mangiferin (Table 9) and phenolic acid p-OH benzoic acid was found to be more in diseased and treated leaves. In healthy leaves gallic acid was found in traces

Bioassay test was carried out with different concentrations of p-OH benzoic acid against fungus *Drechslera specifer*. Colony growth was inhibited at a higher concentration. At 1000 ppm concentration 33.4% of growth inhibited (Fig 4B) Spore germination inhibition was also checked in different concentrations Maximum spore germination inhibition (56%) was noticed at 1000 ppm concentration (Fig. 8A).

var. kesar: The distribution of phytochemicals in healthy, diseased and Gloeosporium Mangiferae treated leaves of var. kesar is presented in Table 5.

The healthy, diseased and fungus treated leaves contained saponins, alkaloids, mangiferin, vanillic acid, syringic acid, p-OH benzoic acid and gallic acid. Healthy leaves contained flavonoid quercetin, where as diseased and

fungus treated leaves were devoid of this flavonol. Diseased and treated leaves contained methylated flavonis like 3'OMe quercetin and 3'4'di OMe quercetin.

Gallic acid in combination with P-OH benzoic acid was tested for their antifungal activity against the fungus. A maximum of 47% colony growth inhibition of the fungus at 1000 ppm concentration was noticed (Fig. 5B). Spore germination was also carried out in different concentrations of these compounds and found 68% spore germination inhibition at high concentration (Fig. 9B).

var. *alphanso*: The distribution of phytochemical compounds in healthy, diseased and *Theilaviopsis paradoxa* treated leaves of var. *alphanso* is presented in Table 6.

The healthy, diseased and treated leaves contained saponins, alkaloids, mangiferin and phenolic acids like vanillic acid, syringic acid, p-OH benzoic acid and gallic acid Beside these healthy leaves contained flavonoids like 3'OMe quercetin, 3'4' di OMe quercetin, which were absent in diseased and treated leaves Diseased and treated leaves contained quercetin only.

Bioassay test was done against the fungus with different concentrations (2,4,6,8 and 10 ml) of plant leachates. At 10 ml concentration of leachates 88.4% of the colony growth was inhibited (Fig. 6A) spore germination was also observed in different concentrations of plant leachates. At 8 ml and 10 ml concentration of leachates 90% and 92% of spore germination was inhibited respectivily (Fig. 10A).

var. *langra*: The distribution of phytochemical compounds in healthy, diseased and fungus treated leaves is presented in Table 7.

The healthy, diseased and *Pestalotiopsis mangiferae* treated leaves contained saponins, alkaloids, flavonoids like quercetin and quercetagetin, mangiferin, syringic acid, p-OH benzoic acid and gallic acid. Concentration of quercetin, mangiferin (Table 9), p-OH benzoic acid and gallic acid was found to be high in diseased and treated leaves as compared to the healthy ones. Gallic acid was found in traces in healthy leaves

Bioassay test was carried out against *P.mangiferae* with quercetin in combination with p-OH benzoic acid at different concentrations. The colony growth was inhibited (65%) at 1000 + 1000 pm concentration of the compound (Fig. 6B) Spore germination inhibition was also checked in different concentrations of test compounds. Maximum spore germination was inhibited (76%) at higher concentration (Fig 10B).

var. *dasheri*: Distribution of phytochemical compounds in healthy, diseased and *chaetomium globosum* treated leaves of var *dasheri* is presented in Table 8.

The compounds like saponins, alkaloids, quercetin, quercetagetin, mangiferin, vanillic acid syringic acid, p-OH benzoic acid and gallic acid were found in healthy, diseased and fungus treated leaves. Apart from these healthy, leaves also contained 3'OMe quercetin. Diseased and treated leaves contained a new phenolic acid, ferulic acid which was absent in healthy ones. Mangiferin concentration (Table 9) and phenolic acid were found to be high in diseased and treated leaves.

Bioassay test with ferulic acid was carried out using different concentrations. At 1000 ppm concentration of ferulic acid 67% of colony growth was inhibited (Fig. 7, Fig. 11).

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Leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy		+			+	+			+	+	+	+	+	<b>+</b> -
Infected		+	_	****	+	+*			+	+**	+*	+*	+*	+*
Treated		+			+	+*			+	+*	+*	+*	+*	+*
1. Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids,														
6.Querce	<ul><li>6.Quercetin, 7. 3'OMe quercetin, 8. 3'4'di OMe quercetin, 9. Quercetagetin,</li></ul>													
10. Mang	giferi	n,	11.	Van	illic	acid,	12	. Sy	ring	ic acid	, 13.	p-OH	I benzo	oic acid,
14. Gallic acid														
+ - indicates present, +* - indicates high concentration,														
indicates absent +** - indicates very high concentration														

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Table 2 : Distribution of phytochemicals in healthy, diseased and Alternaria

alternata treated leaves of M.indica L. var. rajapuri.

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Table 3 : Distribution of	phytochemicals	in	healthy,	infected	and	Curvularia
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Leaves	1	2	3	4	5	6	· 7	8	9	10	11	12	13	14
Healthy		+			+					+	+	+		+
Infected		+			+					+**	+*	+*	<b>.</b>	- <b>-</b> **
Treated	-	+			•				_	+**	+*	+*	<b>+</b> **	+**
1. Tannins	5,	2.	Sap	onin	s,	3.	Proa	inthe	ocya	nidins,	4. I	ridoid	s, 5.A	Jkaloids,
6 Quercetin, 7. 3'OMe quercetin, 8. 3'4'di OMe quercetin, 9. Quercetagetin,														
10 Mangi	ferii	1,	11. '	Vani	llic a	acid		12.	Syri	ngic ac	id, 1	3. p-O	H benz	oic acid,
14 Gallic	acid													
+ - indicates present, indicates absent														
+* - indicates high concentration, +** - indicates very high concentration														

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lunata treated leaves of M.indica L. var. totapuri.

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Leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy		Ŧ			÷	÷			+	+	+	+	+	+ -
Diseased	_	+			÷	+*		-	+	+**	+ <b>*</b>	+*	+**	+*
Treated		+			÷	+*			+	+*	+*	+*	+**	+*
1. Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5 Alkaloids,														
6. Quercet	in, 7	7 3	OM	[e qu	ierce	etin,	83	'4'd	li Ol	Me qu	ercetin	i, 9.Ç	uercet	agetin.
10. Mangil	ferin.	11	l. Va	anilli	c ac	id,	12. 5	Syri	ngic	acid,	13	p-OH	benzoi	c acid.
14 Gallic acid														
indicates present indicates absent indicates in traces														
-* - indicates high concentration +** - indicates very high concentration														

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Table 4 : Distribution of phytochemicals in healthy, diseased and Drechslera

specifer treated leaves of M.indica L. var ladva.

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Leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy		÷			+	+		-	-	+	÷	÷	+	+
Infected		+			+		÷	+		+**	+*	+*	+*	+*
Treated		+			+		<u>_</u>	+		+**	+*	+*	+*	+*
1 Tannins	5,	2. S	apor	ins.		3. P	roar	tho	cyan	idins,	4 Iı	ridoids,	5. A	Alkaloids.

Table 5 : Distribution of phytochemicals in healthy, infected and Gloeosporium

mangiferae treated leaves of M.indica L. var kesar.

6 Quercetin, 7. 3'OMe quercetin, 8. 3'4'di OMe quercetin, 9 Quercetagetin,
10. Mangiferin, 11. Vanillic acid. 12. Syringic acid. 13 p-OH benzoic acid,
14.Gallic acid

## + - indicates present +\* - indicates high concentration

- - indicates absent -\*\* - indicates very high concentration

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Leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy		+			+	_	÷	+		÷	+	+	÷	÷
Diseased		+			+	+*	-		-	+**	+*	+*	+*	<b>_</b> *
Treated		+			÷	+				+**	+*	+*	+*	*
1. Tannins,	2.	Saj	ooni	ns,	3	Proa	ntho	cyar	nidin	is, 4.	Irido	ids.	5 Al	kaloids.
6. Quercet	in, i	7 3	3'01	Me q	uero	cetin	8	3'4'	di C	)Me qu	ierceti	n, 9 (	Querce	tagetin.
10. Mangi	ferin	, 1	1. V	anill	ic ac	cid.	12.	Syri	ngic	acid.	13.,	p-OH	benzo	ic acid,
14 Gallic a	cid													
+ - indicate	s pre	esent	t				-	+* -	indi	icates l	nigh co	oncent	ration	
indicate	indicates absent +** - indicates very high concentration													

Table 6 : Distribution of phytochemicals in healthy, diseased and Thielawopsis

paradoxa treated leaves of M.indica L. var alphanso

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Leaves	1	2	3	4	5 ·	6	7	8	9	10	11	12	13	14	15
Healthy	<u> </u>	+	-		+				· ······	+		+	+	+	- <u></u>
Infected		+			+	*			+	+**		+	+**	+*	- <del></del> -
Treated		+			+	*			+	+*		+	+**	+*	÷
<ol> <li>Tannins,</li> <li>3'OMe</li> </ol>	2. que	Sapc rcetir	onins, n, 8.	3.	. Pro 4'di	antho OMe	ocyar que	nidins rcetir	s, 2 1,	4. Irido 9 Que	vids, 5 ercetag	Alk getin,	aloids. 10	6.Qu Man	iercetin, giferin,
11. Vanillic	e acio	<b>i.</b> 12.	. Syri	ngic	acid	. 13	p-OI	H ber	nzoio	c acid.	14. G	allic a	cid, 15	5. Unic	dentified
+ - indicate	es pr	esent	t		_	- inc	licate	es ab:	sent		1.2	F -)	ndicate	es in t	races —
+* - indic	ates	high	conc	entr	ation		+*	* -	indic	ates ve	ery hig	,h cor	ncentra	tion	

Table 7 : Distribution of phytochemicals in healthy, infected and Pestalotiopsis mangiferae

treated leaves of M.indica L. var. langra.

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Leaves	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Healthy	—	+			+	+	+		+	+	+	+	+	+	
Infected	_	+	-		+	+ <b>*</b>	-		+	+**	+*	+*	+**	+*	+
Treated		+	*****	_	+	+*			+	+*	+*	+*	+**	+*	+
<ol> <li>Tannins</li> <li>3' OMe</li> </ol>	s, 2 que	. Sap	oonir n, 8	is, . . 3`-	3 Pr 4' di	oantł OMe	iocya que	anidi rceti	ns, 4 n.	FI⊓do 9 Que	ids, erceta	5. All getin	caloids	6. Q 0 Ma	)uercetin Ingiferin
11. Vanillio	c acio	1, 12.	. Syr	ingic	acid	. 13.	p-OI	H be	nzoic	acid.	14 G	allic a	cid, 15	5. Fen	ilic acid.
+ - indicate	s pr	esent	t				+*	' - in	dicat	es high	i conc	entra	tion		
– - indicate	s ab	sent					+*	* - ii	ndica	tes ver	y higł	n con	centrat	ion	

Table 8 : Distribution of phyhtochemicals in healthy, diseased and Chaetomium globosum

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treated leaves of M.indica L. var dasheri.

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 Table 9 : Concentration of mangiferin in grams per 100 gms fresh wt. in

 healthy, infected and fungus treated leaves of different

 varieties of M. indica L.

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Varieties	Healthy	Infected	Treated
rajapuri	2 02	2.53	2.29
totapuri	0.84	1.24	1 08
ladva	1 08	2 12	1.25
kesar	1.04	1.82	. 1.78 *
alphanso	1 35	3.06	1 91
langra	1,15	2.73	1.63
dasheri	2.37	3.57	2.74

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Fig. 4 . A - Effect of mangiferin on the colony growth of *Curvularia lunata* at different concentrations (Control, 20ppm, 40ppm, 60ppm, 80ppm & 100ppm)

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 B - Effect of p-OH benzoic acid on the mycelial growth of *Drechslera* specifer C · control, Treatments: 200ppm, 400ppm, 600ppm, 800ppm, 1000ppm



FIG 4

Fig 5 : A - Effect of leachates of *M. indica* leaves inoculated with *Alternaria alternata* on the mycelial growth of same fungus at 2ml, 4ml, 8ml, and 10ml concentrations

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B - Effect of p-OH benzoic acid + Gallic acid on the colony growth of Gloeosportum mangiferate Control, treatments 250ppm, 500ppm, 750ppm & 1000ppm

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Fig: 6: A- Effect of leachate of *Thielaviopsis paradoxa* treated leaves of*M. indica* on mycelial growth of same fungus at different concentations.

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B- Effect of quercetin and p-OH benzoic acid in equal combinations (250ppm, 500ppm, 750ppm and 1000ppm) on the mycelial growth of *Pestalotiopsis mangiferae*.

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FIG.6



Fig. 7: Effect of ferulic acid on colony growth of *Chaetomium globosum* at 200ppm, 400ppm, 600ppm, 800ppm and 1000ppm concentrations.

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FIG.7

Fig. 9: A- Effect of plant leachate on the mycelial growth (0---0) and spore germination (0----0) of *Alternaria alternata* at 2ml, 4ml, 6ml, 8ml, and 10ml concentrations

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B- Effect of p-OH benzoic acid + Gallic acid on mycelial growth
(0----0) and spore germination (0----0) at different concentrations
(250, 500, 750 and 1000 ppm).

Fig. 8: A- Effect of p-OH benzoic acid on myclelial growth (0---0) and spore germination (0----0) of *Drechslera specifer*.
B- Effect of Mangiferin on myclial growth (0---0) and spore

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germination (0----0) of *Curvularia lunata* at different concentrations.



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Fig. 8



Fig. 9

Fig 10 A- Effect of treated leachate on the colony growth (0---0) and spore germination (0---0) of *Thielaviopsis paradoxa* at different concentrations.

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B- Effect of quercetin and p-OH benzoic acid on the mycelial growth (0----0) and spore germination (0----0) of *Pestalotiopsis* mangiferae.

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Fig. 10

Fig. 11: Effect of ferulic acid on the mycelial growth (0---0) of *Chaetomium* globosum at 200ppm, 400ppm, 600ppm, 800ppm and 1000ppm concentrations.

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Fig. 11

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Phytochemical changes in healthy, malformed and *F. moniliforme* treated inflorescences of different varieties of *M.indica* L.

var *rajapuri*<sup>•</sup> The distribution of the phytochemical compounds in healthy, malformed and *F* monulaforme treated inflorescences of var *rajapuri* is presented in Table 10

The healthy, malformed and *F. monuliforme* treated inflorescences of var *rajapuri* contained flavonol such as quercetin, phenolic acids like vanillic acid, syringic acid, gallic acid and ferulic acid, tannins, saponins, proanthocyanidins, steroids and mangiferin Apart from these the malformed and fungus treated inflorescences showed the presence of phenol and p-OH benzoic acid The concentration of quercetin, gallic acid and ferulic acid was found to be higher in malformed and fungus treated inflorescences. The concentration of mangiferin (Table 17) and p-OH benzoic acid was found to be very high in malformed panicles Steroids were found to be same in all the three types inflorescences studied.

The fungitoxicity of p-OH benzoic acid was assayed by inoculating the pathogen in a medium containing of different concentrations (200, 400, 600, 800 and 1000 ppm) The bioassay test with p-OH benzoic acid showed the restriction of colony growth at the maximum concentration of the test compound. The cololy growth was inhibited to 80% at 1000 ppm concentration (Fig 12A) Spore germination was also checked with different concentrations of p-OH benzoic acid Maximum spore germination was inhibited (89%) in 1000 ppm concentration (Fig 16A)

var. *totapuri* The distribution of phytochemical compounds present in healthy, malformed and *F. moniliforme* treated panicles is presented in Table 11

The healthy, malformed and fungus treated panicles contained tannins, saponins and proanthocyanidins The healthy panicles showed the presence of quercetin, 3' OMe quercetin, 3'4' di OMe quercetin, mangiferin, phenolic acids like vanillic acid, p-OH benzoic acid, gallic acid and ferulic acid and steroids. The malformed and *F. moniliforme* treated panicles showed the same compounds as that of healthy ones, except quercetin. Quercetin was absent in malformed and fungus treated inflorescences The concentration of p-OH benzoic acid, ferulic acid and mangiferin (Table 17) was found to be more in malformed and fungus treated panicles as compared to healthy ones

The leachates was subjected to bioassay test with F. moniliforme. The leachates showed maximum inhibition (80%) of colony growth at 8 ml concentration (Fig 12B) Spore germination was also checked with different concentrations and found maximum inhibition at 8 ml concentration (Fig. 16B) var *ladva*. The distribution of phytochemical compounds of healthy, malformed and F. moniliforme treated inflorescences is presented in Table 12.

Healthy, malformed and treated panicles contained tannins, saponins, proanthocyanidins, quercetin, mangiferin, vanillic acid, syringic acid and gallic acid. Besides these compounds healthy panicles also contained 3'4' di OMe quercetin Malformed and treated panicles contained 3' OMe quercetin, p-OH benzoic acid and ferulic acid. The concentration of quercetin, mangiferin (Table 17) and gallic acid was found to be high in malformed and treated panicles

The fungitoxicity of quercetin and ferulic acid was assayed by inoculating F *Monthforme* in a medium containing combination of quercetin and ferulic acid. The colony growth was inhibited by 55 5% at 1000 + 1000

ppm concentration (Fig 13A). Spore germination was also checked with different concentrations of quercetin and ferulic acid in combination. The spore germination was inhibited by 26% at 250 + 250 ppm, 37% at 500 + 500 ppm, 48% 750 + 750 ppm and 70% at 1000 + 1000 ppm concentration of test compounds (Fig. 17A).

var. *kesar*: The distribution of phytochemical compounds in healthy, malformed and *F. moniliforme* treated panicles is presented in Table 13.

The healthy panicles contained tannins, saponins, proanthocyanidins, quercetin, 3'OMe quercetin, mangiferin and phenolic acids like vanillic acid, syringic acid, p-OH benzoic acid and gallic acid. Malformed and F. moniliforme treated panicles were also contained same phenolic acids except ferulic acid. Ferulic acid was absent in healthy panicles. Apart form these, malformed and fungus treated panicles contained only 3'4' di OMe quercetin. Quercetin and 3' OMe quercetin were absent in malformed and treated panicles. Steroids were found to be same in all three types studies. The concentration of mangiferin (Table 17), p-OH benzoic acid and gallic acid was found to be more in malformed and treated ones.

Bioassay test was carried out against *F. moniliforme* with ferulic acid in different concentrations. Colony growth was inhibilited by 31.5% at 1000 ppm  $\mathcal{S}/$ concentration of ferulic acid (Fig. 13B). Spore germination was also observed in different concentrations of ferulic acid. At 750 ppm and 1000 ppm concentration of ferulic acid 39% and 47% inhibition of spore germination was observed respectively (Fig. 17B).

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var. *alphanso*: The distribution of phytochemical compounds in healthy, malformed and fungus treated panicles of var. *alphanso* is presented in Table 14

The healthy panicles contained tannins, saponins, proanthocyanidins, 3' OMe quercetin, kaempferol, mangiferin and gallic acid. Malformed and *F. moniliforme* treated panicles showed the presence of tannins, saponins, proanthocyanidins, quercetin, mangiferin, vanillic acid, p-OH benzoic acid, gallic acid and ferulic acid The concentration of mangiferin (Table 17), p-OH benzoic acid and gallic acid was found to be very high in malformed and treated inflorescences as compared to healthy ones.

The antifungal nature of p-OH benzoic acid and ferulic acid was assayed by inoculating *F. monultforme* in a medium containing p-OH benzoic acid and ferulic acid combination The colony growth was inhibited by 81.3% at 1000 ppm concentration (Fig. 15A) Spore germination was also observed in different ppm concentrations of p-OH benzoic and ferulic acid. The maximum inhibition (88%) was observed in 1000 ppm concentration (Fig. 18**Å**).

var. *langra*: The distribution of phytochemical compounds in healthy, malformed and *F. monuluforme* treated inflorescences is presented in Table 15

The healthy inflorescences contained tannins, saponins, proanthocyanidins, 3' OMe quercetin, 3'4' di OMe quercetin, mangiferin, vanillic acid, syringic acid, gallic acid and ferulic acid Malformed and treated panicles contained same phenolic acids found in healthy ones, except ferulic acid which was absent in malformed and treated inflorescences. Malformed and treated panicles also showed the presence of quercetin which was absent in

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healthy ones 3' OMe quercetin and 3'4' di OMe quercetin were found absent in malformed and treated ones The concentration of quercetin, mangiferin (Table 17) and gallic acid was found to be high in malformed and treated panicles.

Biossay test was carried out against *F. moniliforme* with quercetin. Maximum colony growth inhibition (35 5%) was observed at 1000 ppm (Fig 14A) Chromatogram biossay using quercetin as test compound also showed inhibition zone on chromatogram (Fig. 14B) Spore germination inhibition was found to be 69% at 1000 ppm concentration (Fig. 18**B**). var. *dasheri* : The distribution of phytochemical compounds in healthy, malformed and treated panicles of var *dasheri* is presented in Table 16.

Healthy, malformed and *F. moniliforme* treated inflorescences of var *dasheri* showed the presence of tannins, saponins, proanthocyanidins, flavonols like 3' OMe quercetin, mangiferin and phenolic acids like vanillic acid, syringic acid, p-OH benzoic acid and gallic acid. Apart from these healthy panicles also contained ferulic acid. The concentration of 3' OMe quercetin, mangiferin and phenolic acids was found to be high in malformed and treated panicles as compared to healthy ones.

Biossay test was carried out with mangiferin. Mangiferin showed the maximum colony growth inhibition of *F. moniliforme* (53.2%) at 40 ppm concentration (Fig 15B) Maximum spore germination inhibition (62%) was also found at 40 ppm concentration (Fig 19).

1	2	3	4	5	6	7	8	9	10	11	12	13	14
+	+	+	–	—	+		+	+,	+		+-	+	+
+	+	+		-	+*	+	+**	÷	+	+**	+*	+*	
+	+	+	-	-	+*	+	+*	+	+	+**	+*	+*	4
	1 + + +	1 2 + + + + + +	1 2 3 + + + + + + + +	1 2 3 4 + + + - + + + - + + + -	$\begin{array}{cccccccccccccccccccccccccccccccccccc$								

Table 10. Distribution of chemical compounds in healthy, malformed andF. moniliforme treated inflorescences of M. indica L. var. rajapuri.

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Tannins, 2. Saponins, 3 Proanthocyanidins, 4. Iridoids, 5. Alkaloids,
Quercetin, 7 Phenol, 8 Mangiferin, 9. Vanillic acid, 10. Syringic acid,
p-OH benzoic acid, 12. Gallic acid, 13. Ferulic acid, 14 Steroids

+ - indicates present + - indicates in traces +\* - indicates high concentration -- indicates absent +\*\* - indicates very high concentration

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Table 11. Distribution of phytochemicals in healthy, malformed and treated inflorescences of *M. indica* L var *totapuri*.

Inflorescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy ,	+	+	+	•		+	+	+	+	 -		+	+-	+
Malformed	+	+	÷	-	-	-	+	+	+*	+	-	+*	+*	+*
Treated	+	+	+	-	-	-	+	+	+*	+	-	+*	+*	+*
				-				****				000000000000000000000000000000000000000	*****	

Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids, 6.Quercetin,
7 3' OMe quercetin, 8 3'4'di OMe quercetin, 9. Mangiferin, 10. vanillic acid, V/
11. Syringic acid, 12. p-OH benzoic acid, 13. Gallic acid, 14 Ferulic acid.
+ - indicates present +<sup>-</sup> - indicates in traces +\* - indicates high concentration
- - indicates absent +\*\* - indicates very high concentration

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Table 12 Distribution of chemical compounds in healthy, malformed andF. momliforme treated inflorescences of M. indica L. var.ladva

Inflorescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy	+	+	+		<del></del>	+	-	+	+	+	+	-	+-	
Malformed	+	+	+			+*	+		+**	+	÷	+	+*	+
Treated	+	+	+		-	+*	+	-	+*	+	+	+	<b>÷</b> *	+

Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids. 6 Quercetin,
3' OMe quercetin, 8 3'4' di OMe quercetin, 9 Mangiferin, 10. Vanillic acid.
Syringic acid, 12. p-OH benzoic acid, 13 Gallic acid, 14. Ferulic acid

+ -	indicates present	<u>+* -</u>	indicates high concentration

-- indicates absent -\*\* - indicates very high concentration

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Table 13 : Distribution of phytochemicals in healthy, malformed andF. moniliforme treated inflorescences of M. indica L. var kesar

Inflorescences	l	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Healthy	+	+	+	_		+	-		+	+	+	+	+		+
Malformed	+	÷	+		_	_	-	+	+**	÷	+	+*	+*	+	+
Treated	+	+	÷		_	_	-	÷	+*	+	+	+*	+*	+	+
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Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids. 6 Quercetin,
3' OMe quercetin, 8. 3'4'di OMe quercetin, 9 Mangiferin, 10 Vanillic acid,
11 Syringic acid, 12.p-OH benzoic acid, 13. Gallic acid, 14 Ferulic acid, 15. Steroids
+ - indicates present -- indicates absent +<sup>-</sup> - indicates in traces
+\* - indicates high concentration -\*\* - indicates very high concentration

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Table 14	Distrib	ution	L	of	pl	hyto	chen	nica	ls	in	heal	thy	<sub>ک</sub> ma	lforn	ned	and
	F. mo	mlifo	rme	treat	ed i	inflo	resce	ence	es of	ГM.	india	ca Ĺ.	var.	alpł	hanso	, ,
Inflores		1 2		4	 		7	8	0	10	11	12	13			16

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mnorescences		-	5	-1	2	Ū	•	U	-	10		12	15	14	1.2	10
Healthy	+	+	+		-	-	+		+		-		+	-	+	+
 Malformed	+	ł	+		-	+			+**	+	-	+*	+*	+	+	
Treated	+	+	+			+		-	+*	÷		+*	+*	+	+	
AND	00000000						1000000000									

Tannins. 2 Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids. 6.Quercetin,
7 3' OMe quercetin, 8. 3'4' di OMe quercetin, 9 Mangiferin, 10. Vanillic acid,
11. Syringic acid, 12. p-OH benzoic acid, 13. Gallic acid, 14 Ferulic acid,
15 Steroids, 16. Kaempferol
+ - indicates present -- indicates absent +<sup>-</sup> - indicates in traces
+\* - indicates high concentration +\*\* - indicates very high concentration

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Table 15 Distribution of chemical compounds in healthy, malformed and treated

Inflorescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy	+	+	+			_	+	+	+	+	+	-	+	+
Malformed	÷	+	+			+*	-	-	+**	+	+		+*	
Treated	+	+	+	-	-	+*	-	-	+*	+	+	-	+*	

inflorescences of M. indica L. var. langra.

Tannins. 2. Saponins, 3 Proanthocyanidins, 4. Iridoids, 5. Alkaloids. 6.Quercetin,
7 3' OMe quercetin, 8. 3'4' di OMe quercetin, 9. Mangiferin, 10. Vanillic acid.
11. Syringic acid, 12. p-OH benzoic acid, 13 Gallic acid, 14. Ferulic acid.
+ - indicates present -- indicates absent +<sup>-</sup> - indicates in traces
+\* - indicates high concentration +\*\* - indicates very high concentration

Table16: Distribution of phytochemicals in healthy malformed and treated  $\bigcirc$  inflorescences of M. indica L. var. dasheri

Inflorescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Healthy	+	+	+				, + ,		+	+		+	+	+
Malformed	+	+	+	-	-		+*	-	+**	+*		+*	+*	-
Treated	+	+	+	-		-	+*		+*	+*	·	+*	+*	
	0000000000	000000000								, 1			ويهيب وسلط فخط	anterrocommon

Tannins, 2. Saponins, 3. Proanthocyanidins, 4. Iridoids, 5. Alkaloids, 6.Quercetin,
7. 3' OMe quercetin, 8. 3'4' di OMe quercetin, 9. Mangiferin, 10.Vanillic acid,
11. Syringic acid, 12. p-OH benzoic acid, 13. Gallic acid, 14. Ferulic acid.
+ - indicates present -- indicates absent +- indicates traces
+\* - indicates high concentration +\*\*\* indicates very high concentration

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Varieties	Healthy	Malformed	Treated
rajapuri	0.37	1 13	0.66
totapuri	0.26	0 69	0.36
ladva	0 29	0.81	0.51
kesar	0.26	1.03	0 54
alphanso	0.37	1.07	0.56
langra	0.37	0 87	0.57
dasheri	0.58	1.66	0 69

Table17: Concentration of mangiferin in mg / 100 g fr. wt. in healthy, malformed and *F.moniliforme* treated inflorescences of different varieties of *M*. *indica* L.

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Fig. 12: A- Effect of p-OH benzoic acid on the mycelial growth of *Fusarium moniliforme*. (Control, Treatment : 200ppm, 400ppm, 600ppm, 800ppm, 1000ppm).

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B- Effect of leachate of inflorescence of *M. indica* inoculated with *Fusarium moniliforme* on mycelial growth of same fungus at 2ml, 4ml, 6ml, and 8ml concentrations.

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## FIG.12

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Fig. 13: A- Effect of quercetin and p-OH benzoic acid on the mycelial growth of *Fusarium moniliforme* at different concentrations.

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B- Effect of ferulic acid on mycelial growth of *Fusarium moniliforme* at 250ppm, 500ppm, 750ppm and 1000ppm concentrations.

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FIG 13

Fig. 14 : A - Effect of quercetin on mycelial growth of *Fusarium moniliforme* at 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm concentrations.

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B -Chromatogram bioassay showing inhibition of *l'usarium* moniliforme.

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FIG.14

Fig. 15: A- Effect of ferulic acid + p-OH benzoic acid on mycelial growth of *Fusarium moniliforme*. Control, Treatments : 250ppm, 500ppm, 750ppm and 1000ppm.

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 B- Effect of mangiferin on the mycelial growth of *Fusarium* moniliforme at 20ppm, 40ppm, 60ppm, 80ppm and 100ppm concentrations.

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FIG .15

- Fig. 16: A- Effect of p-OH benzoic acid on mycelial growth (0-----0) and spore germination (0-----0) of *I-usarium monuliforme* at different concentrations.
  - B- Effect of treated leachate on mycelial growth (0----0) and spore germination (0----0) of *Fusarium moniliforme* at 2ml, 4ml, 6ml and 8ml concentrations.

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Fig. 16

Fig. 17: A- Effect of quercetin and p-OH benzoic acid on colony growth (0----0) and spore germination (0----0) of Fusarium moniliforme.

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B- Effect of ferulic acid on mycelial growth (0---0) and spore germination (0----0) of *Fusarium moniliforme* at 250ppm, 500ppm, 750ppm and 1000ppm.

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Fig. 17

Fig. 18: A- Effect of quercetin on mycelial growth (0----0) and spore germination (0----0) of *Fusarium moniliforme* at different concentrations

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B-Effect of p-OH benzoic acid and ferulic acid in combination on mycelial growth (0----0) and spore germination (0----0) of *Fusarium moniliforme*.

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Fig. 18

Fig. 19: Effect of mangiferin on mycelial growth (0---0) and spore germination (0----0) of *I-usarium monuliforme* at 20ppm, 40ppm, 60ppm, 80ppm and 100ppm concentrations.

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Fig. 19

## **BIOCHEMICAL CHANGES**

Post-infectional biochemical changes in ascorbic acid content, peroxidase activity, total soluble sugars, total phenols and mangiferin content in the leaves of different varieties of *M. indica* L.

Ascorbic Acid Content : In comparison to healthy leaves, diseased leaves and fungus treated leaves showed considerable reduction in the ascorbic acid content in all the varieties of *M. induca* studied. Fungus treated leaves showed less reduction in ascorbic acid content as compared to naturally infected leaves. Mangiferin treated leaves showed increased quantity of ascorbic acid as compared to healthy ones (Table 18)

**Peroxidase Activity :** Peroxidase activity increased with the severity of infection. The maximum activity of peroxidase was observed in severely infected leaves in all varieties. Fungus treated leaves also showed increased activity of peroxidase. Whereas mangiferin treated leaves showed decreased activity of the enzyme as compared to healthy ones (Table 19)

Total Soluble Sugars : There was a dicline in the total soluble sugars in infected and fungus treated leaves Infected leaves contained less sugar content as compare to fungus treated ones. Mangiferin treated leaves also showed the dicline in sugar content as compared to healthy leaves (Table 20).

**Total Phenols :** The results (Table 21) showed that there was an increase in total phenol contents in infected, fungus treated and mangiferin treated leaves of all varieties as compared to the healthy leaves Naturally infected leaves contained more total phenols than fungus treated leaves. Mangiferin treated leaves also showed more amount of total phenols

**Mangiferin Content :** The results showed that there was an increase in mangiferin content in infected, fungus treated and mangiferin treated leaves of all the varieties. Increase in mangiferin content was more in mangiferin treated leaves as compared to infected and fungus treated leaves (Table 22).

Biochemical changes in healthy malformed, fungus treated and mangiferin treated inflorescences of different varieties of *M. indica* L.

Ascorbic Acid Content : The results showed that there was decline in ascorbic acid content in malformed and fungus treated inflorescences of all varieties Decline was more in fungus treated inflorescences as compared to malformed panicles. Mangiferin treated inflorescence showed increased amount of ascorbic acid content as compared to healthy ones (Table 18).

**Peroxidase Activity :** Data clearly indicated that malformed and fungus treated inflorescences showed high peroxidase activity. Fungus treated panicles showed more peroxidase activity as compared to malformed panicles. Mangiferin treated panicles showed less enzyme acitvity as compared to healthy ones (Table 19).

**Total Soluble Sugars :** There was a decline in total sugar contents in malformed and fungus treated inflorescences. Fungus treated panicles showed less amount of total sugars as compared to malformed panicles. Mangiferin treated panicles also showed the reduction in their total sugar content (Table 20).

**Total Phenols :** In comparison to healthy, malformed, fungus treated and mangiferin treated panicles contained more amount of total phenols. Malformed panicles contained relativily more total phenols as compared to fungus treated and mangiferin treated (Table 21).

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**Mangiferin Content :** The results (Table 22) showed higher mangiferin content in malformed, fungus treated and mangiferin treated panicles. Malformed panicles showed high concentration of mangiferin. Mangiferin treated panicles also showed increased amount of mangiferin as compred to healthy and fungus treated panicles

Changes in ascorbic acid, peroxidase activity, total soluble sugars, total phenols and mangiferin content in healthy, infected, fungus treated and mangiferin treated fruits of different varieties of *M. indica* L.

Ascorbic Acid : The results showed that there was a decline in ascorbic acid content in infected and fungus treated fruits of all varieties. Decline was more in infected fruits as compared to fungus treated ones. There was an enhancement in ascorbic acid content after the treatment of mangiferin as compared to healthy ones (Table 18)

**Peroxidase Activity :** Present data showed that there was an increase in peroxidase activity in infected and fungus treated fruits as compared to healthy ones, mangiferin treated fruits showed less peroxidase activity (Table 19).

Total Soluble Sugars : Data showed that there was decrease in total soluble sugar content in infected, fungus treated and mangiferin treated fruits. Decline was more in infected and fungus treated as compared to mangiferin treated ones (Table 20)

**Total Phenols :** Infected, fungus treated and mangiferin treated fruits contained more phenolic contents as compared to healthy ones. Phenolic content was more in infected as compared to fungus treated and mangiferin treated fruits (Table 21)

Mangiferin Content : The results (Table 22) showed that mangiferin content was found to be more in infected, fungus treated and mangiferin treated fruits as compared to healthy ones. Mangiferin treated fruits showed relatively high mangiferin content than infected and fungus treated ones.

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## Table 18 : Changes in ascorbic acid content ( mg g<sup>-1</sup> fr. wt. ) in healthy, infected, fungus

treated and mangiferin treated leaves, inflorescences and fruits of different

	rajapuri	totapuri	ladva	kesar	alphanso	langra	dasheri
Leaves							
Healthy	1.83	4 61	2 53	1 38	1 46	1 78	1.66
Infected	0.93	2 55	0 94	1.03	1.00	1 18	0.64
Fungus treated	1.18	3 55	1.97	1 33	114	1 56	1 45
Mangiferin treated	3 10	5.50	2.82	1.64	1.73	1 94	2 25
Inflorescences							
Healthy	1.00	1.26	0 56	071	1 1 1	1 05	1.05
Malformed	0 82	1.02	0 37	0.59	1.00	0 96	0.91
Fungus treated	0 55	0 90	034	0.53	0.73	0 93	0 82
Mangiferin treated	1.36	1.52	0 98	0.99	1 29	1.15	1.37
Fruits							
Healthy	02	0.14	0.22	0.2	1 05	141	0.36
Infected	0.14	01	019	0.13	0.5	0.47	0.23
Fungus treated	0.19	0.12	0 21	0.18	0 73	1.24	0 29
Mangiferin treated	0.23	0.17	0 28	0.22	1.23	1.47	0.41

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varieties of M. indica.

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## Table 19 : Changes in peroxidase activity ( unit min<sup>-1</sup> mg<sup>-1</sup> fr. wt. ) in healthy, infected, fungus treated and mangiferin treated leaves, inflorescences and fruits of different varieties

of M. indica L.

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	rajapuri	totapuri	ladva	kesar	alphanso	langra	dasheri
Leaves							
Healthy	0.0027	0 0023	0 0036	0 0012	0 0034	0 0019	0 0093
Infected	0.0114	0.0123	0 0079	0.0108	0.0183	0.0232	0.0186
Fungus treated	0.0105	0.0092	0.0062	0.0091	0.0126	0.0109	0 0143
Mangiferin treated	0.0016	0.0018	0 002	0.0009	0.0018	0.0013	0.0017
Inflorescences							
Healthy	0.0024	0.0033	0.0038	0.0032	0.005	0.0025	0 0042
Malformed	0.0026	0.0040	0.0040	0.0046	0 0057	0 0031	0 0058
Fungus treated	0.0030	0.0047	0 0046	0 0072	0 0067	0 0062	0.0089
Mangiferin treated	0.0019	0.0028	0.0025	0.0026	0.0027	0 0023	0 0037
Fruits							
Healthy	0.0074	0.0064	0.0071	0 0114	0 0064	0 0036	0.0060
Infected	0 0094	0 0084	0.0147	0.014	0.0107	0.0159	0.0130
Fungus treated	0.0075	0.0067	0.0079	0.0124	0 0067	0.0078	0 0083
Mangiferin treated	0 0064	0 0021	0 0063	0 0086	0.0032	0 003 1	0 0053

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# Table 20 : Changes in total soluble sugar content ( mg g<sup>-1</sup> dry. wt. ) in healthy, infected, fungus treated and mangiferin treated leaves, inflorescences and fruits of different varieties of M. indica L.

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	rajapuri	totapuri	ladva	kesar	alphanso	langra	dasheri
Leaves							
Healthy	15.87	23.50	14 88	22.10	17 14	30.74	22 12
Infected	10 62	11.45	08 62	09.51	08.07	10 17	12 82
Fungus treated	11 63	16.98	11 90	11.27	12.78	18 68	15.87
Mangiferin treated	13.40	18.37	14 15	12.39	15.10	23.76	19 02
Inflorescences							
Healthy	45.60	39.85	46 89	53.96	36.37	42.75	42.46
Malformed	40 73	32.28	32.73	42.46	32.28	39.21	37.16
Fungus treated	30 08	24 55	27 42	35.61	21 89	28.81	27 76
Mangiferin treated	42 68	36.04	34.94	48 21	37 59	40 03	38 26
Fruits							
Healthy	391.01	473 28	305 2	421.31	425 95	346.34	401.40
Infected	207 89	274.23	148.17	294 14	272.03	190 86	220.72
Fungus treated	278.66	370.00	258 75	341 69	327.09	232 22	298.78
Mangiferin treated	298.12	417 99	294.14	363 37	343 46	285 29	335.72

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### Table 21 : Changes in total phenols (mg g<sup>-1</sup> fr. wt.) in healthy, infected, fungus treated and mangiferin treated leaves, inflorescences and fruits of different varieties of M. indica L.

	rajapuri	totapuri	ladva	kesar	alphanso	langra	dasheri <sup>,</sup>
Leaves							
Healthy	42.82	66.23	65 19	58 92	28 18	70.19	73 36
Infected	81.35	100 13	90.74	78.64	38 59	104 82	110.87
Fungus treated	64 15	85.53	73.53	77 70	34 42	86.73	92.31
Mangiferin treated	52.67	74 05	68.32	63.10	32.33	79 2 <b>7</b>	82.40
Inflorescences							
Healthy	116.82	125.94	108.47	137 31	133.82	124 64	130 25
Malformed	188 68	152.95	147.50	220 13	208.08	173.66	222.42
Fungus treated	15436	146 54	135.07	170 79	162.19	158.02	170 01
Mangiferin treated	132.98	138.72	120 47	161 14	143.93	139.76	154.94
Fruits							
Healthy	08.17	04 75	12.15	10.37	10.17	9.74	9.43
Infected	09 17	05.94	15.25	11.15	10.53	10 16	11.22
Fungus treated	09 43	10.79	17.24	14.87	13.61	12.83	13.56
Mangiferin treated	8 50	06.26	12.98	12.05	12.98	11.16	12.56

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## Table 22 : Changes in mangiferin content (g/100g fr. wt.) in healthy, infected, fungus treated and mangiferin treated leaves, inflorescences and fruits of different varieties of

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	rajapuri	totapuri	ladva	kesar	alphanso	langra	dasheri
Leaves							
Healthy	1 3 5 1	0 831	1 169	0 941	1.298	1.228	1.392
Infected	1 851	1 380	1.528	1.799	1 581	1.758	1.871
Fungus treated	1 606	1 179	1.214	1 450	1 690	1 526	1.818
Mangiferin treated	1 978	1.791	1.631	1.862	2.135	2.570	2.334
Inflorescences							
Healthy	0 508	0 342	0.414	0 381	0.473	0 449	0 671
Malformed	1 131	1 305	1.213	1 373	1.663	1.458	1.764
Fungus treated	0 907	0.572	0.718	0 659	0 872	0 791	0.989
Mangiferin treated	1 057	0.788	0.720	0 948	0.951	0 899	1.138
Fruits					•		
Healthy	0 286	0 377	0.355	1.263	0 419	0 331	0 857
Infected	0 328	0 563	0.439	1.740	0.541	0.395	0.996
Fungus treated	0 342	0.632	0.524	2.17	0.609	0 524	1.151
Mangiferin treated	0 372	0.865	0.603	2.514	0 831	0 628	1 324
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M. indica L.

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#### **Anatomical studies**

Leaves - Transverse sections of leaves showed the germination of some fungi like Alternaria alternata (Fig 20A,C), Gloeosportum mangiferae (Fig. 20B) and Pestalotiopsis mangiferae (Fig. 20D) It also showed the presence the inter and intracellular hyphae of Alternaria alternata (Fig. 22E, F; Fig. 20E, F). Transverse sections of leaves revealed the histological variations between healthy and infected leaves. A relatively thick cuticle (19 43 µm, Table 23) covered the epidermis of infected leaves. The cuticle often extended into the radial walls of the adjacent epidermal cells (Fig. 21B) Sub epidermal region was occupied by 3-4 layered thick walled lignified sclereids in healthy while in r.le diseased leaves such regular arrangement of sclerieds was not found (Fig. 21A, B) A relatively thick walled sclerenchymatous perivascular fibers (Fig. 21C, D) covered the gum resin duct in the cortical region of the infected leaves. Gumresin duct lumen was filled with secretory material whereas in healthy leaves it was free from any such deposition (Fig. 21E, F). Dark deposits were commonly found in the intercellular spaces and in phloem parenchyma (Fig. 22B). Such deposition was not noticed in healthy leaves (Fig. 22A) The vessel element walls were relatively thick and lignified (Fig. 22C, D) and the lumen showed accumulation of granular darkly stained material (Fig. 22D). The frequency of vessels was found to be more but there diameter was less (Table 23). In healthy leaves the vessels were relatively thin walled with no dark deposition in the lumen. (Fig. 22 c).

#### **Histochemical studies**

**Proteins**: Diseased leaves showed more accumulation of protein content in the mesophyll tissue (Fig. 23B) as compared to healthy ones (Fig. 23Å)

Starch: In comparison to diseased leaves, starch accumulation was more in healthy ones. Starch grains were more in the ground parenchyma of the midrib region (Fig. 25A, B) Spongy parenchymatous cells also showed starch grains in healthy leaves.

**Phenolics**: Phenolic contents were more accumulated in the diseased leaves particularly at the site of infected region (Fig. 20B). Deposites of phenolics were also noticed in the lumen of vessel elements whereas the healthy vessel elements were free from any deposition (Fig. 24A, B)

**Wood** - The gross structure of the xylem collected from the infected region of the main trunk remained similar to that of normal xylem (Fig 26A, B). However, the sections from infected xylem were darkly stained due to the accumulation of dark brown Phenolic contents in the cells These contents were mostly found localized more in axial and ray parenchyma cells (Fig 27A, Fig. 28D) The infected and uninfected xylem were separated by a transition zone which was lightly stained than the infected xylem (Fig 27A) The accumulation of phenolic contents was more in the region between the infected and transition zone (Fig. 27A) The infected region showed the association of fungal hyphae with the parenchyma cells and vessels (Fig 26B, D). The hyphae often formed a net-work in ray parenchyma cells, probably passing from one cell to the next, through the cell walls (Fig. 26D). Such hyphal association was not found with the elements of normal xylem (Fig. 26C) Fungal hyphae was also noticed along the cell walls and lumen of vessel elements (Fig 26B). In the infected region the vesssels were also noticed with tyloses completely occupying the lumen (Fig 27B) On the other hand, vessel lumen diameter was found to be less and their frequency was more compared to that of normal xylem (Table 23)

#### **Histochemical Studies**

Starch Considerable variation was noticed in the distribution of starch in the parenchyma cells of infected and normal xylem. The axial and ray parchchyma cells in the normal xylem showed heavy accumulation of starch grains (Fig. 28A). While in the infected xylem, both the cells were either completely devoid of starch and if present it was scarce (Fig. 28B).

**Phenolics:** Infected xylem showed higher accumulation of Phenolic compounds as compared to the normal one Phenolic contents appeared in diffuse form and occupy entire lumen of the ray cells (Fig. 28C, D). The transition zone between infected and non infected xylem i.e. fibers, vessel elements, axial and ray parenchyma were completely filled with darkly stained Phenolic compounds (Fig. 27A) In the normal xylem Phenolic contents were rarely noticed and found sparsey distributed in ray and axial parenchyma cells (Fig. 28C)

*********	***************************************	Η	I
1.	Leaf cuticle thickness	14.74 μm	19.43 μm
		± 0.39	±063
2.	Vessel frequency in leaves	11.44	12 17
		± 3.92	± 2 82
3.	Vessel lumen diameter in leaves	16.90	16.36
		± 5 36	± 4.23
4.	Vessel frequency in wood	3.12	10.24
		± 0.74	±444
5.	Vessel lumen diameter in wood	29.34	12.78
		± 5.41	± 2.54
6.	Gum - resin duct lumen diameter in leaf	45 68	36 12
		± 17 73	± 10 9

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Table 23: Anatomical variations in healthy (H) and infected (I) leaves and wood of Mangifera indica L var. rajapuri

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Fig. 20: A-F show transverse sections of diseased leaves.

- A- Conidia of Alternaria alternata germinating on epidermal surface of lamina (arrow) × 250.
- B- Fruiting body of the fungus *Gloeosporium* on lamina (arrow). Note the accumulation of phenolic contents at the site of infection × 375.
- C- Lower epidermis of the midrib region showing lesion and spore of *Alternaria* (arrow). Note lignified cells in the mesophyll surrounded by cells containing phenolic contents × 375.
- D- Conidium of *Pestalotiopsis* germinating on epidermis of midrib region (arrow). Note the depression resulted by the germinating conidium on the host epidermis  $\times$  700.
- E-Intracellular hyphal fragments in mesophyll cells of the midrib  $(arrow) \times 700$ .
- F- Intracellular hyphal fragments in spongy parenchyma cells of the lamina (arrow)  $\times$  280.

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FIG .20

- Fig. 21: Transverse sections of the healthy (A, C and E) and diseased leaves (B, D and F).
  - A- Thin cuticle covering the epidermis (arrow head). Note thick walled lignified 3-4 layered sclereids (arrows) below the epidermis × 240.
  - B- Thick cuticle extending into radial walls of the epidermal cells (arrow head). Note the irregularly placed sclereide cells (1-2 layered) below the epidermis (arrows) × 400
  - C- Note the relatively thin walled (arrows) perivascular fibres in the mesophyll region of midrib  $\times$  240.
  - D- Relatively thick walled perivascular fibres (arrows) in mesophyll region of midrib × 240.
  - E- Resin-duct lumen devoid of any secretion (arrow) in the mesophyll region of midrib  $\times$  300.
  - F- Resin-duct lumen filled with secretion (arrows) in the mesophyll region of midrib  $\times$  300.

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FIG.21

- Fig. 22: Transverse sections of healthy (A and C) and diseased (B, D, E and F) leaves.
  - A- Phloem tissue at midrib region showing scanty phenolic contents (arrows)×300.
  - B- Phloem tissue in midrib region showing densely stained cells with phenolic contents (arrows) × 300.
  - C- Lumen of vessel elements free from any deposites (arrows) in the midrib region × 300
  - D- Lumen of vessel elements filled with grannular densely stained bodies (arrows) in the midrib region  $\times$  300.
  - E- Intercellular hyphae in the spongy parenchyma (arrows) of the lamina  $\times$  300.
  - F- Enlarged view of Fig. 'E' showing a portion of spongy parenchyma with intercellular hyphae × 875.

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FIG.22

Fig. 23: Transverse sections of healthy (A) and diseased (B) leaves.

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A- Healthy leaf showing scanty distributions of protein bodies in hypodermal cells × 360

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B- Infected leaf showing accumulation of protein bodies in hypodermal cells (arrows) × 420.



FIG. 23

Fig. 24: Transverse sections of healthy (A) and diseased (B) leaf midrib of *M. indica* L. var. *rajapuri*.

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A- Vessel elements (V) showing no accumulation of phenolic content  $\times$  390.

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B- Accumulation of phenolic content in the lumen of a vessel elements (arrows) × 1000.

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FIG.24

Fig. 25: Transverse sections of healthy (A) and diseased (B) leaves.

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A- Midrib ground tissue showing accumulation of starch grains (arrows) and resin ducts free of secretion (arrow head)  $\times 250$ .

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B- Gum-resin duct lumen showing positive reaction for resin secretion (arrow head) and ground tissue devoid of starch grains (arrows)× 300.

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FIG.25

- Fig. 26: Transverse (A, B) and radial longitudinal (C, D) sections of secondary xylem of *M. indica* var. *rajapuri*.
  - A- Normal xylem with a solitary vessel, ray parenchyma, axial parenchyma and fibres. Note the elements free of fungal association × 160.
  - B- Infected xylem showing mycelial mat in the lumen of a vessel element. Note the transversly cut portion of hyphae in the adjacent parenchyma cells (arrows)  $\times$  480.
  - C- Ray parenchyma cells free from fungal pathogen  $\times$  420.
  - D- Ray parenchyma cells from infected xylem showing fuungal mat in the cell lumen and along the cell walls  $\times$  440

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FIG 26

Fig. 27: Radial longitudinal (A) and transverse (B) sections of secondary xylem from infected wood.

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A- Heavy accumulation of phenolic contents in the xylem elements bordering the transition zone (arrows) × 300.

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B- Vessel filled with tyloses (arrows) blocking the lumen  $\times$  300.



FIG . 27

- Fig. 28: A-D. Radial longitudinal sections of xylem of Mangifera indica var. rajapuri
  - A- Normal secondary xylem showing the distribution of total pholysaccharides in axial (arrows) and ray (arrow heads) parenchyma cells × 200.
  - B- Infected xylem free of total polysaccharides in axial (arrow) and ray (arrow head) parenchyma cells × 200.
  - C- Normal xylem with scanty distribution of phenolic contents in ray parenchyma cells (arrows) × 200.
  - D- Secondary xylem from infected region showing heavy accumulation of phenolics in ray parenchyma cells (arrows)× 200

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FIG. 28