Experimental

4.1. Pathogenicity test of *C. gloeosporioides* in different brinjal varieties

Pathogenicity of *C. gloeosporioides* was tested following detached leaf inoculation technique as well as whole plant inoculation technique (Plate V and Plate VI). Experiments following the above mentioned two techniques were performed on 28 brinjal varieties viz. Pusa purple long, Pusa purple round, Pant brinjal-4, Orissa green, Green round, Ppl-74, Suchitra variety, BE-706, Supriya variety, Nisha variety, Pant rituraj, Green long, PK-123, BSS-465-chhaya, Arka keshav, Kanha hybrid, BSS-330, Agrayani variety, Nirupama variety, BSS-470 janam, Pant samrat, Baromasi local, Muktakeshi local, Kuroi variety, Aam begun, Preeti variety, Lalguli variety and Shamala variety. The disease assessment procedures and details of incubation periods and temperatures etc. have been mentioned in the materials and methods (Section 3.7).

4.1.1. Pathogenicity test following detached leaf inoculation technique

Pathogenicity of *C. gloeosporioides* was performed on detached leaves of 28 different varieties of brinjal as mentioned above following technique described under materials and methods (Section 3.6.1). The results presented in the Table 3 represents percentage lesion formed and mean diameter of lesions after 24, 48 and 72 hours of incubation. Graphical representation of percent lesion formed after 72 h has been shown in Fig. 3. Spore suspension droplets were mounted on the leaves and the infected sites showed typical black sunken anthracnose lesions (Plate V). The lesions were counted and the diameters of the lesions were measured. The percentage of lesions formed in three separate experiments (i.e. replicates) were calculated. The mean values of the three experiments were tabulated in Table 3.

On the basis of the data presented in the Table 3 and Fig. 3, it is evident that Pusa purple long, Pusa purple round, Pant brinjal-4, Orissa green and Green round were susceptible to *C. gloeosporioides* because they produced 94.93%, 93.66%, 91.17%, 90.50% and 90.17% lesions respectively. A variety was considered highly susceptible when it produced more than 80% lesion and resistant when the lesion production was less than 25% after 72 h of incubation. Accordingly, Shamala variety, Lalguli variety, Preeti variety and Aam begun were resistant and produced 16.33%, 17.50%, 22.50% and 23.83% lesions respectively.

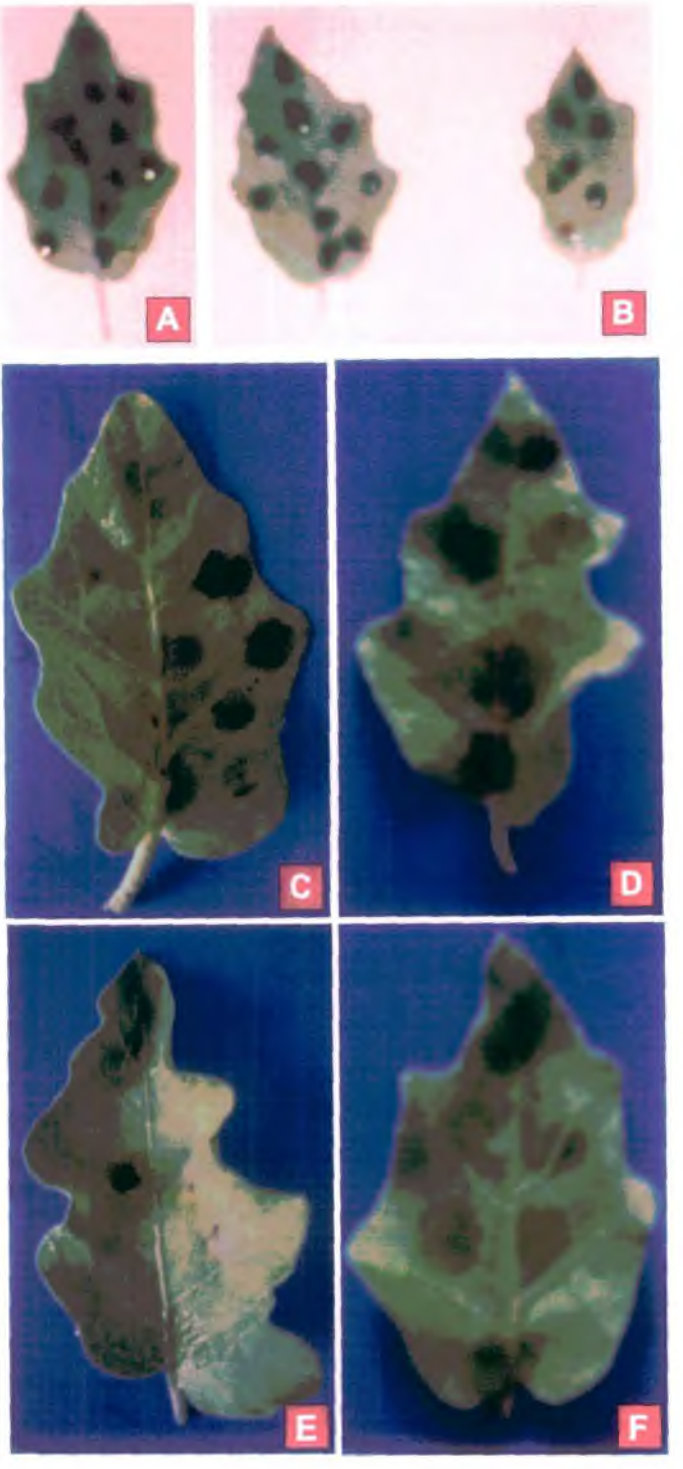

Plate V : Leaves of susceptible brinjal varieties artificially infected with *Colletotrichum gloeosporioides* following detached leaf inoculation technique after 72 h of inoculation

Fig. A: Pusa purple long, **Fig. B:** Pusa purple round, **Fig. C:** Arka keshav, **Fig. D:** Pant rituraj, **Fig. E:** Shamala variety, **Fig. F:** Pusa purple long (control).

Table 3: Pathogenicity test of *Colletotrichum gloeosporioides* on detached leaves of different brinjal varieties

Brinjal varieties	*Percentage of lesion formed			**Mean diameter of lesion (mm)		
	Incubation period (Hours)			Incubation period (Hours)		
	24	48	72	24	48	72
Pusa purple long	35.27±1.56	83.33±1.86	94.93±1.27	1.82±0.09	6.27±0.19	10.87±0.34
Pusa purple round	31.83±1.62	79.83±1.92	93.66±1.59	1.75±0.05	5.81±0.26	10.43±0.38
Pant brinjal-4	30.83±1.13	74.00±1.53	91.17±1.48	1.80±0.10	6.25±0.18	10.71±0.21
Orissa green	30.20±1.54	73.00±1.15	90.50±1.26	1.72±0.08	5.48±0.19	9.97±0.38
Green round	29.13±1.78	72.33±1.20	90.17±1.69	1.52±0.12	5.25±0.18	9.43±0.22
Ppl-74	27.43±1.58	69.17±1.69	85.67±1.45	0.90±0.10	5.08±0.19	9.03±0.23
BE-706	27.06±0.64	68.83±1.09	85.17±1.36	0.85±0.08	5.05±0.18	8.67±0.19
Supriya variety	24.20±1.10	65.17±1.17	84.17±1.36	0.77±0.09	4.83±0.12	8.40±0.15
Nisha variety	23.67±1.02	64.5±1.50	83.83±1.42	1.00±0.08	4.63±0.15	7.07±0.24
Suchitra variety	20.93±2.11	59.50±1.26	81.33±1.76	1.03±0.08	4.08±0.20	6.67±0.32
Pant rituraj	20.16±0.84	55.67±1.20	78.83±1.59	1.37±0.07	3.65±0.13	5.77±0.27
Green long	16.97±0.78	55.50±0.76	77.50±1.26	0.98±0.08	4.02±0.16	5.85±0.28
PK-123	15.86±1.05	50.67±1.20	74.33±1.59	1.52±0.04	3.93±0.19	6.02±0.24
BSS-465-chhaya	15.20±1.31	49.83±1.01	73.83±1.09	1.03±0.11	3.53±0.12	5.62±0.16
Arka keshav	13.03±0.86	47.83±1.17	72.50±1.76	1.08±0.07	2.72±0.16	5.05±0.33
Kanha hybrid	12.80±0.85	43.50±1.15	67.17±1.17	1.00±0.05	3.62±0.16	5.35±0.18
BSS-330	9.60±0.45	43.50±1.04	58.00±1.76	0.98±0.06	2.48±0.09	4.52±0.22
Agrayani variety	7.87±0.47	42.50±1.04	56.83±1.17	0.87±0.08	2.88±0.16	5.28±0.29
Nirupama variety	0	35.50±1.04	53.17±1.17	0	2.17±0.11	4.17±0.21

Contd.Table 3

Table 3 (Contd.....)

Pathogenicity test of *Colletotrichum gloeosporioides* on detached leaves of different brinjal varieties

Brinjal varieties	*Percentage of lesion formed			**Mean diameter of lesion (mm)		
	Incubation period (Hours)			Incubation period (Hours)		
	24	48	72	24	48	72
BSS-470 janam	0	29.83±0.60	51.83±1.48	0	3.22±0.14	4.43±0.22
Pant samrat	0	28.33±0.88	50.33±1.48	0	2.55±0.13	4.35±0.13
Baromasi local	0	0	39.67±1.86	0	0	4.05±0.15
Muktakeshi local	0	0	35.67±1.76	0	0	3.52±0.14
Kuroi variety	0	0	35.00±1.53	0	0	3.42±0.20
Aam begun	0	0	23.83±1.01	0	0	3.17±0.16
Preeti variety	0	0	22.50±1.04	0	0	2.85±0.13
Lalguli variety	0	0	17.50±0.87	0	0	2.48±0.20
Shamala variety	0	0	16.33±0.44	0	0	2.40±0.13
CD at 5%	0.72	0.59	0.61	0.05	0.12	0.15

*Mean of three replications. Data after ± represent standard error values.

** Average of 150 lesions.

4.1.2. Pathogenicity test following whole plant inoculation technique

The whole plant inoculation method as described by Dickens and Cook (1989) was applied. Pathogenicity of *C. gloeosporioides* was further tested on whole brinjal plants of 28 different varieties as mentioned earlier. The methodologies and disease assessment procedure are described in materials and methods (Section 3.6.2 and 3.7.2 respectively) and results are summarized in Table 4.

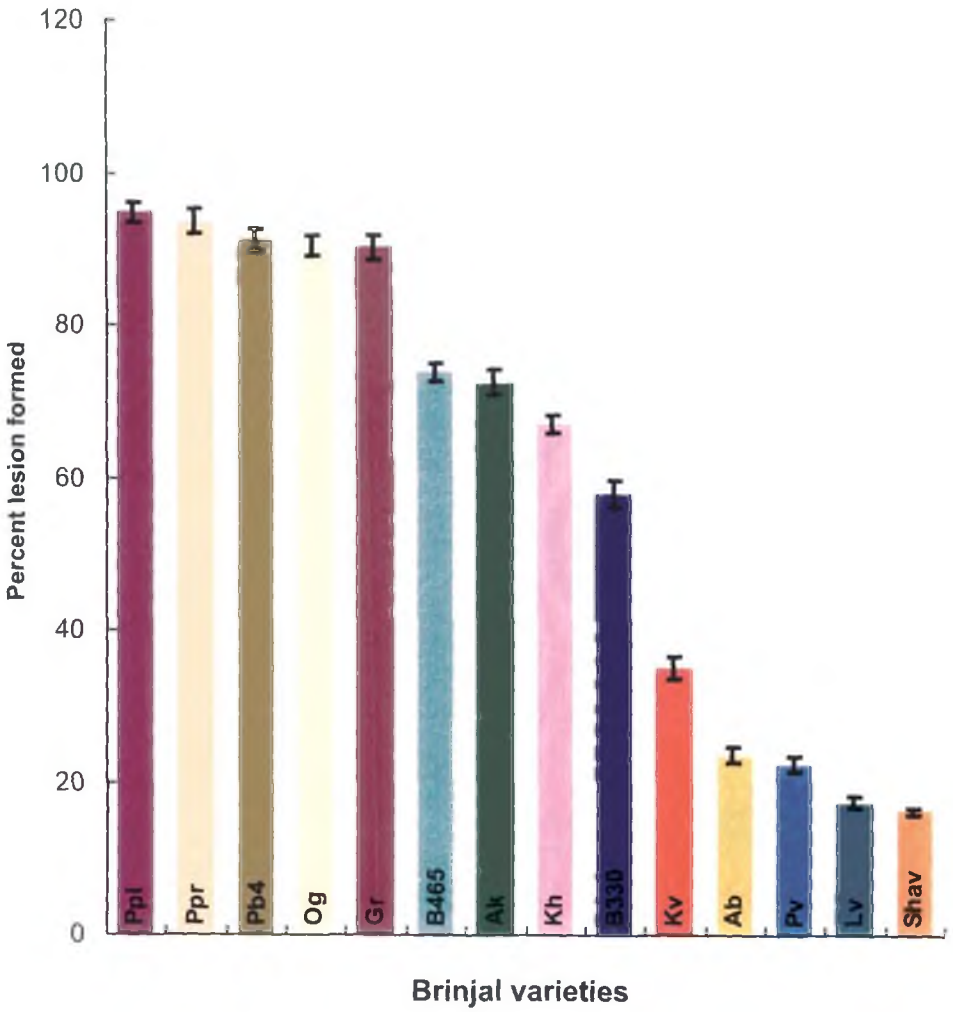

Fig. 3 : Disease developement (percent lesion formed) after 72 h caused by *Colletotrichum gloeosporioides* in some selected brinjal varieties.

Abbreviations:

Ppl = Pusa purple long	Kh = Kanha hybrid
Ppr = Pusa purple round	B330 = BSS-330
Pb4 =Pant brinjal-4	Kv = Kuroi variety
Og = Orissa green	Ab = Aam begun
Gr = Green round	Pv = Preeti variety
B465 = BSS-465-chhaya	Lv = Lalguli variety
Ak = Arka keshav	Shav = Shamala variety

Table 4: Pathogenicity test of *C. gloeosporioides* on whole plant of different brinjal varieties

Brinjal varieties	Incubation period (Days)							
	3		6		9		12	
	Mean disease index/ plant*	Mean no. of lesions / Plant	Mean disease In dex / Plant	Mean no. of lesion/ Plant	Mean disease index/ plant	Mean no. of lesions/ plant	Mean disease index/ plant	Mean no. of lesions/ plant
Pusa purple long	2.56 ±0.25	15.06 ±0.45	7.22 ±0.32	20.50 ±0.51	12.83 ±0.58	27.15 ±0.98	17.20 ±0.62	30.72 ±0.65
Pusa purple round	2.40 ±0.23	14.92 ±0.40	6.69 ±0.31	19.20 ±0.49	12.76 ±0.47	26.27 ±0.77	17.15 ±0.55	30.57 ±0.53
Pant brinjal-4	2.38 ±0.24	14.87 ±0.41	6.03 ±0.20	19.38 ±0.56	11.35 ±0.77	25.16 ±0.77	14.43 ±0.73	29.27 ±0.67
Orissa green	2.02 ±0.19	13.45 ±0.38	5.56 ±0.22	17.82 ±0.24	10.00 ±0.48	21.80 ±0.60	14.50 ±0.44	28.61 ±0.61
Green round	1.79 ±0.25	11.52 ±0.12	5.30 ±0.17	17.67 ±0.61	9.83 ±0.44	20.87 ±0.70	13.50 ±0.57	27.77 ±0.56
Ppl-74	1.47 ±0.20	8.97 ±0.38	4.57 ±0.24	17.57 ±0.58	6.43 ±0.58	19.80 ±0.98	12.76 ±0.39	24.55 ±0.78
BE-706	1.45 ±0.23	7.75 ±0.18	3.62 ±0.21	13.76 ±0.44	4.50 ±0.33	13.70 ±0.47	9.48 ±0.54	20.10 ±0.95
Supriya variety	1.03 ±0.15	5.83 ±0.31	2.79 ±0.17	12.08 ±0.52	3.88 ±0.35	14.98 ±0.60	7.77 ±0.47	18.15 ±0.78
Nisha variety	1.00 ±0.08	5.78 ±0.27	2.46 ±0.13	11.14 ±0.47	3.87 ±0.29	13.77 ±0.69	6.43 ±0.45	16.42 ±0.59
Suchitra variety	0.83 ±0.87	5.60 ±0.21	2.30 ±0.15	10.22 ±0.44	2.93 ±0.24	12.54 ±0.39	6.44 ±0.42	16.42 ±0.46
Pant rituraj	0.60 ±0.07	5.20 ±0.28	2.42 ±0.09	9.82 ±0.34	2.90 ±0.20	10.60 ±0.60	6.11 ±0.40	18.05 ±0.68
Green long	0.58 ±0.05	4.93 ±0.25	2.25 ±0.15	8.95 ±0.52	2.89 ±0.17	10.60 ±0.50	5.42 ±0.41	16.33 ±0.76
PK-123	0.57± 0.03	4.35 ±0.18	2.09 ±0.15	8.13 ±0.57	2.67 ±0.24	9.95 ±0.28	5.58 ±0.20	15.62 ±0.52
BSS-465 chhaya	0.51 ±0.06	4.32 ±0.19	2.00 ±0.13	7.16 ±0.50	2.66 ±0.24	10.28 ±0.50	5.55 ±0.13	15.73 ±0.61
Arka keshav	0.51 ±0.05	4.30 ±0.18	2.28 ±0.12	7.41 ±0.39	2.63 ±0.18	10.01 ±0.60	5.10 ±0.26	14.79 ±0.47
Kanha hybrid	0.39 ±0.05	3.55 ±0.09	1.94 ±0.15	7.33 ±0.30	2.58 ±0.17	10.15 ±0.38	5.07 ±0.19	13.88 ±0.38

Contd. Table 4

Table 4 (Contd....) Pathogenicity test of *C. gloeosporioides* on whole plant of different brinjal varieties.

Brinjal varieties	Incubation period (Days)							
	3		6		9		12	
	Mean disease index/ plant*	Mean no. of lesions / Plant	Mean disease In dex / Plant	Mean no. of lesion/ Plant	Mean disease index/ plant	Mean no. of lesions/ plant	Mean disease index/ plant	Mean no. of lesions/ plant
BSS-330	0.38 ±0.04	3.47 ±0.15	1.82 ±0.11	6.93 ±0.38	2.57 ±0.21	12.11 ±0.45	5.40 ±0.37	18.58 ±0.53
Agrayani variety	0.30 ±0.04	2.68 ±0.14	1.22 ±0.17	6.92 ±0.46	2.59 ±0.16	13.26 ±0.52	5.10 ±0.25	17.07 ±0.53
Nirupama variety	0.26 ±0.03	2.47 ±0.14	1.31 ±0.19	6.86 ±0.41	2.57 ±0.18	12.23 ±0.37	5.09 ±0.20	14.95 ±0.54
BSS-470 *janam*	0.18 ±0.05	2.22 ±0.11	1.02 ±0.14	5.36 ±0.45	2.45 ±0.23	11.87 ±0.34	4.90 ±0.18	13.66 ±0.56
Pant Samrat	0.17 ±0.02	2.08 ±0.09	1.01 ±0.13	5.20 ±0.35	2.01 ±0.16	9.93 ±0.43	4.84 ±0.11	15.30 ±0.53
Baromasi local	0	0	0.90 ±0.08	6.18 ±0.30	1.95 ±0.15	10.40 ±0.35	4.78 ±0.14	12.70 ±0.47
Muktakeshi local	0	0	0.88 ±0.07	5.29 ±0.24	1.94 ±0.12	8.27 ±0.44	4.35 ±0.28	12.50 ±0.38
Kuroi variety	0	0	0.77 ±0.12	5.53 ±0.34	1.57 ±0.22	7.57 ±0.42	4.17 ±0.23	13.33 ±0.74
Am begun	0	0	0.72 ±0.09	4.42 ±0.25	1.56 ±0.18	7.40 ±0.36	4.14 ±0.12	10.67 ±0.51
Preeti variety	0	0	0.54 ±0.05	3.94 ±0.23	0.94 ±0.13	6.47 ±0.38	3.27 ±0.19	9.76 ±0.51
Lalguli variety	0	0	0	0	0.86 ±0.11	5.33 ±0.18	2.81 ±0.17	8.54 ±0.35
Shamala variety	0	0	0	0	0.66 ±0.13	5.05 ±0.10	2.45 ±0.13	8.18 ±0.44
CD at 5%	0.09	0.14	0.09	0.23	0.18	0.28	0.20	0.37

*Mean of 3 replications. Data after ± represent standard error values.
** Average of 50 lesions.

From the data represented on the Table 4 and Fig. 4, it was quite clear that Pusa purple long variety showed maximum disease development (mean disease index / plant was 17.20) after 12 days of inoculation. Therefore brinjal plants of Pusa purple long variety were considered as the most susceptible variety towards the pathogen among the varieties tested. Varieties like Pusa purple round, Pant

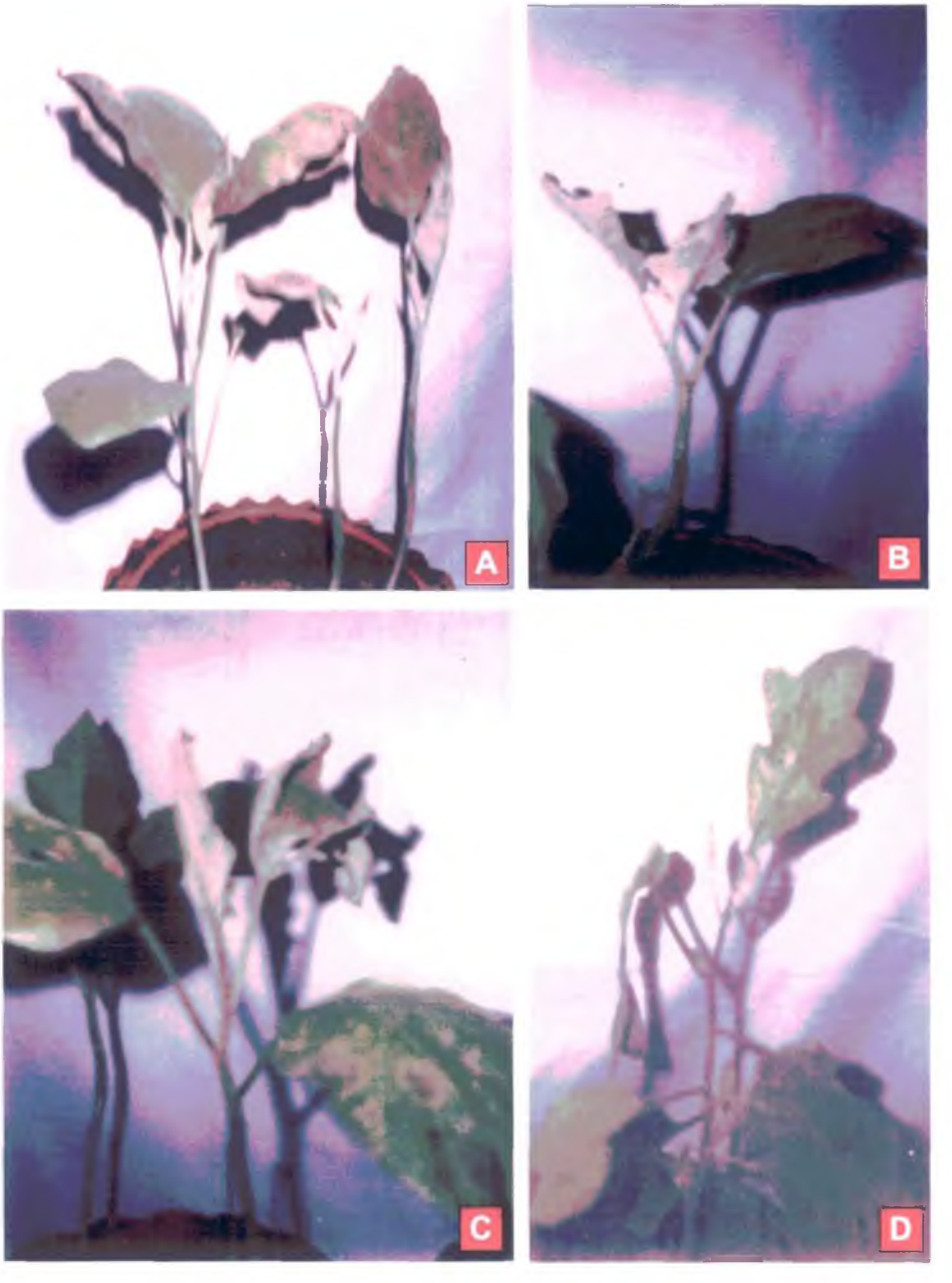

Plate VI : Diseased plants of susceptible brinjal varieties artificially infected with *C. gloeosporioides* following whole plant inoculation techniques.

Fig. A : Pusa purple long **Fig. B :** Orissa green

Fig. C : Pusa purple round **Fig. D :** BE-706.

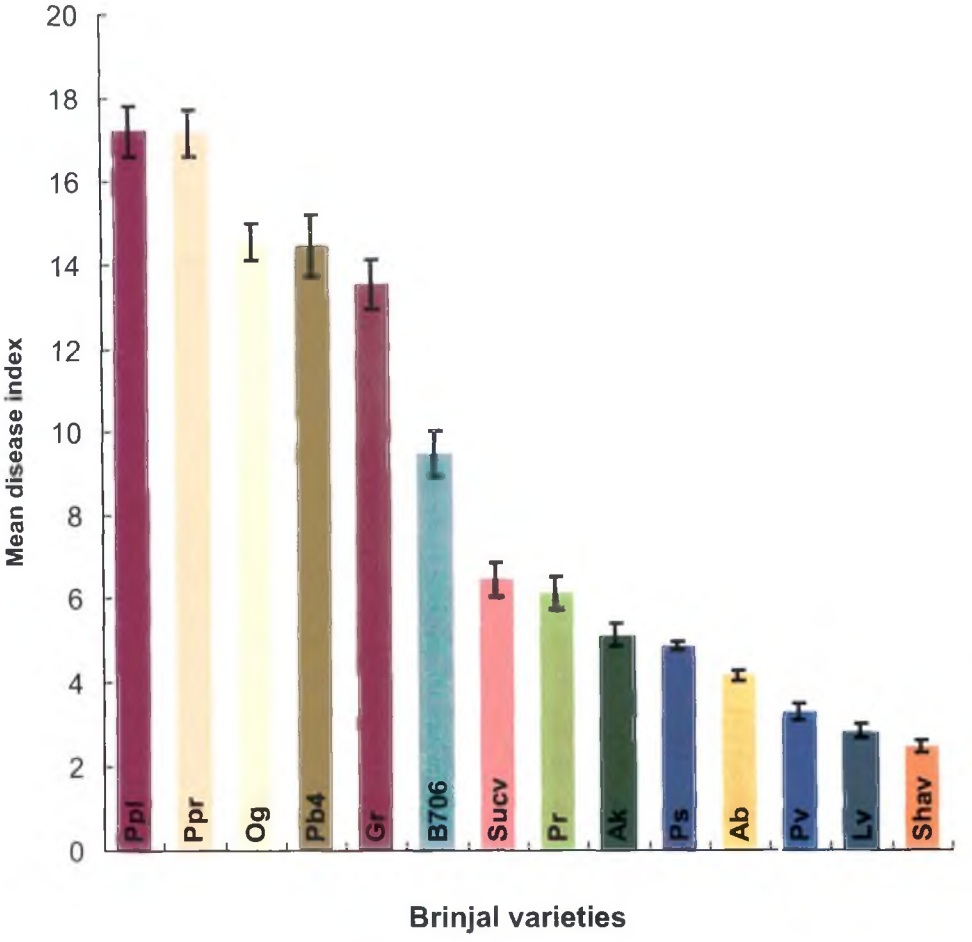

Fig. 4 : Mean disease index after 12 days of inoculation by *Colletotrichum gloeosporioides* in some brinjal varieties.

Abbreviations:

Ppl = Pusa purple long	Pr = Pant rituraj
Ppr = Pusa purple round	Ak = Arka keshav
Og = Orissa green	Ps = Pant samrat
Pb4 =Pant brinjal-4	Ab = Aam begun
Gr = Green round	Pv = Preeti variety
B706 = BE-706	Lv = Lalguli variety
Sucv = Suchitra variety	Shav = Shamala variety

brinjal-4, Orissa green and Green round were also considered as susceptible as their mean disease index / plant values were 17.15, 14.43, 14.50 and 13.50 respectively after 12 days of inoculation. On the other hand Shamala variety was the most resistant as it produced minimum value of mean disease index / plant (2.45) among the varieties tested. Similarly, Lalguli variety, Preeti variety and Aam begun were considered resistant as they produced 2.81, 3.27 and 4.14 values of mean disease index / plant respectively after 12 days of inoculation.

4.2. Observation of morphology of *C. gloeosporioides*

The morphology of the pathogen *C. gloeosporioides* was observed in PDA and OMA (slants and plates) and PDB (Erlenmeyer flask). When the fungus was cultured in PDA or PDB, the mycelia was white in colour, which gradually turned pale yellow and further darker to gray (Plate VII). In OMA, mycelia were pale yellow coloured but growth was not as profuse as PDA. Huge masses of pinkish acervuli were produced in OMA, which was very less when grown in PDA.

For microscopic observations, mycelia were taken in microscopic slides from pure culture and stained using cotton-blue in lactophenol. The slides were mounted with cover glass, sealed and observed under microscope. Mycelia and conidia of the fungus were light colored. The length and breadth of the mature conidia were 13-16 µm and 4-6 µm respectively. The mature conidia were light, one-celled and hyphae were septate, the diameter of the mature hyphae was between 3-5 µm (Fig. 5, Plate VII A-E & Plate VIIIA).

4.3. Culture conditions affecting growth and sporulation of *C. gloeosporioides*

A detailed study on *in vitro* growth and sporulation of *C. gloeosporioides* was conducted under different culture conditions in several solid and liquid media. The details of the study are recorded in the following pages.

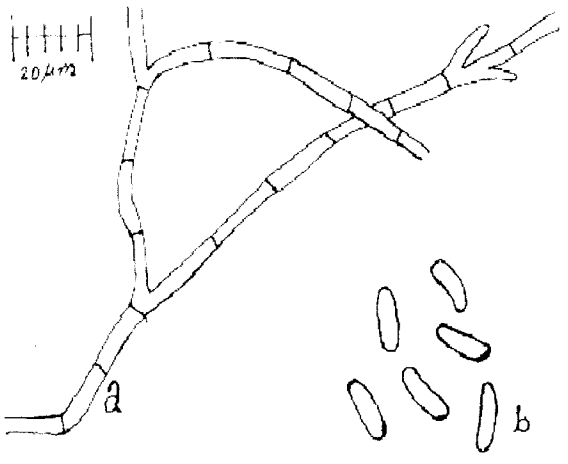

Fig. 5 : a. Hypha of *C. gloeosporioides* with septa and branching.
b. Spores of *C. gloeosporioides.*

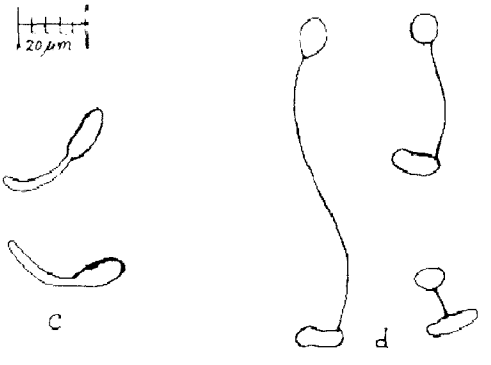

Fig. 5 : c. Germinating spores with germ tubes.
d. Germinated spores with appressoria formed.

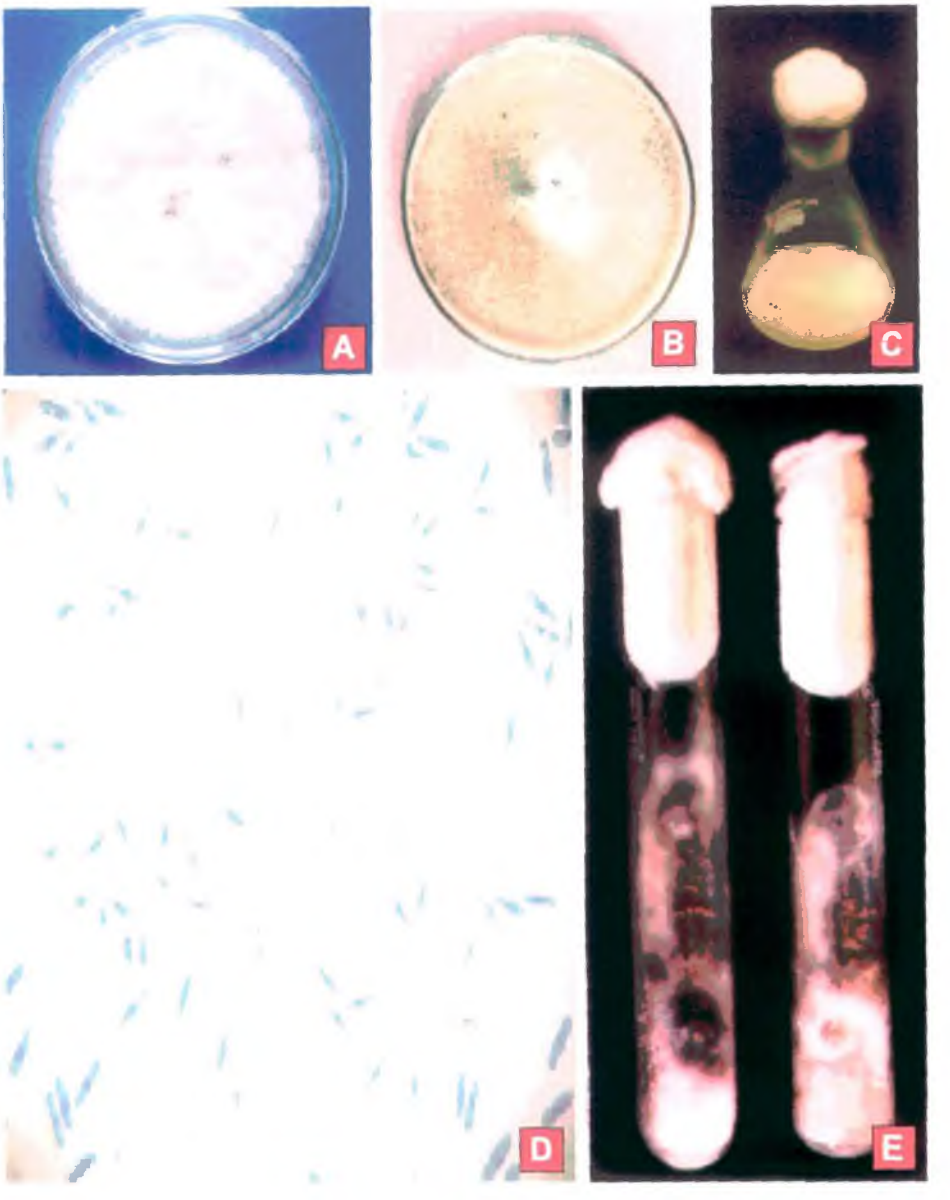

Plate VII

Fig. A : Culture of *Colletotrichum gloeosporioides* in PDA.

Fig. B : Sporulated culture of *C. gloeosporioides* in OMA.

Fig. C : Culture of *C. gloeosporioides* in liquid medium (PDB).

Fig. D : Mature spores of *C. gloeosporioides*.

Fig. E : Culture of *C. gloeosporioides* in slants.

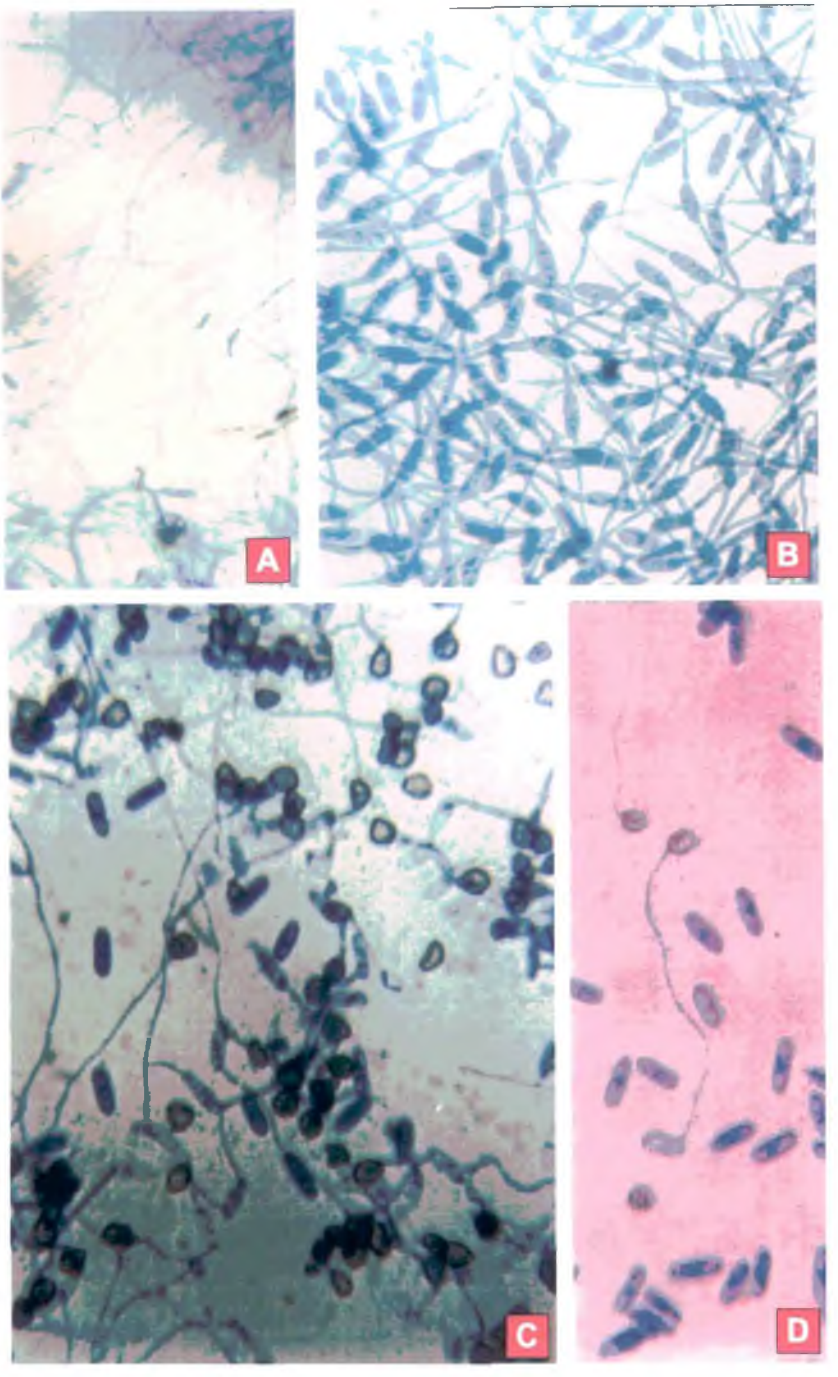

Plate VIII

Fig. A : Hyphae of *Colletotrichum gloeosporioides*.

Fig. B : Germinated spores.

Fig. C & Fig. D : Germinated spores with appressoria.

4.3.1. Mycelial growth of *C. gloeosporioides* in different solid media

In order to assess the vegetative growth of *C. gloeosporioides* in solid media, nine different media viz. potato dextrose agar (PDA), oat meal agar (OMA), leaf extract agar (LEA), Czapek Dox agar (CDA), Richards's agar (RA), yeast extract mannitol agar (YEMA), malt extract agar (MEA), potato carrot agar (PCA) and nutrient agar (NA) were used. Experiments were performed in three replications. Detailed procedure is given under materials and methods (Section 3.8.2). The radial growth of the fungus was measured and noted in Table 5.

Table 5: Mycelial growth and sporulation of *Colletotrichum gloeosporioides* in different solid media.

Medium of growth	Radial growth (mm)* and sporulation (Days)									
	1		2		3		4		5	
	Growth	Spn**	Growth	Spn	Growth	Spn	Growth	Spn	Growth	Spn
PDA	14.17 ± 1.09	–	31.17 ± 0.93	–	45.83 ± 1.42	–	56.50 ± 1.04	–	70.00 ± 0.00	–
OMA	10.83 ± 0.93	–	22.83 ± 0.83	+	35.83 ± 1.09	++	44.83 ± 1.36	+++	54.50 ± 1.04	++++
LEA	15.00 ±0.76	–	33.17 ±1.17	–	50.50 ±1.04	–	60.83 ±0.93	–	70.00 ± 0.00	–
CDA	10.33 ± 0.73	–	23.17 ± 0.60	–	36.83 ± 0.60	–	48.67 ± 0.88	–	59.67 ± 1.59	+++
RA	10.00 ± 0.44	–	21.83 ± 0.60	–	38.67 ± 0.88	–	55.50 ± 0.86	–	70.00 ± 0.00	–
YEMA	9.83 ± 0.67	–	22.00 ± 0.76	+	34.17 ± 1.09	++	46.00 ± 1.26	++ +	51.00 ± 1.00	++++
MEA	10.17 ± 0.67	–	22.33 ±0.93	–	32.83 ±1.17	–	47.17 ± 1.36	–	58.50 ± 1.04	–
PCA	11.17 ± 0.93	–	26.00 ± 1.26	–	36.17 ±1.09	–	46.50 ± 0.76	–	56.17 ± 1.17	–
NA	8.17 ± 0.60	–	19.00 ± 1.00	–	26.83 ±0.83	–	34.17 ± 0.93	+	41.33 ± 1.01	++
CD at 5%	0.83		1.59		1.87		1.95		1.60	

*Mean of three replications. Data after ± represent standard error values.
Pinkish masses of spore colony formed.
**Spn = Sporulation, – = Nil, + = poor, ++ = fair, +++ = good, ++++ = excellent.

From Table 5 and Fig. 6, it was evident that *C. gloeosporioides* can grow in all the media tested but LEA, PDA and RA showed best growth of the fungus. They showed 70 mm radial growth (maximum measurable growth in 70 mm petridish) after 5 d of inoculation. Sporulation was found to occur in various media but huge masses of pinkish acervuli were found in OMA and YEMA.

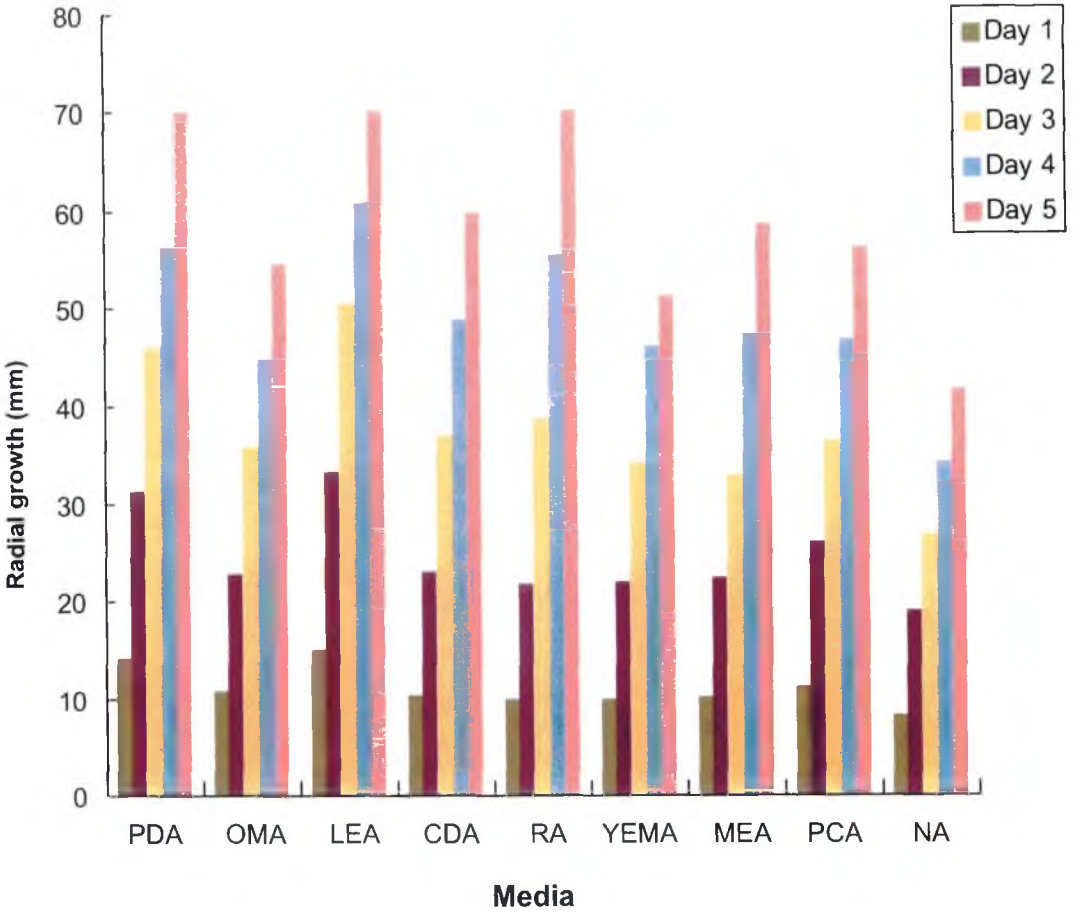

Fig. 6 : Mycelial growth of *Colletotrichum gloeosporioides* in different solid media.

Abbreviations : PDA = Potato dextrose agar
OMA = Oat meal agar
LEA = Leaf extract agar
CDA = Czapek Dox agar
RA = Richard's agar
YEMA = Yeast extract mannitol agar
MEA = Malt extract agar
PCA = Potato carrot agar
NA = Nutrient agar

4.3.2. Mycelial growth of *C. gloeosporioides* after different periods of incubation

The growth of *C. gloeosporioides* was assessed after different periods of incubation at 28±1 °C. The test fungus was inoculated in sterile PDB, OMB and RM (50 ml in 250 ml Erlenmeyer flask) taken in triplicates according to the method described in section 3.8.2. After 5, 10, 15, 20 and 25 d of incubation, the fungal mycelia were harvested and strained through muslin cloth, blotted and dried at 60 °C. Finally the dried mycelia were cooled and mycelial dry weights were taken. The results are summerized in Table 6 and Fig. 7.

Table 6. Growth of *C. gloeosporioides* after different incubation periods in different liquid media.

Medium of growth	Mycelial dry weight (mg)* after incubation period (Days)				
	5	10	15	20	25
PDB	220.00 ± 2.08	300.00 ±2.52	420.00 ± 3.06	490.00 ± 3.60	455.00 ± 1.53
OMB	155.00 ±2.51	200.00 ± 2.08	270.00 ± 2.08	310.00 ±3.01	280.00 ± 2.08
RM	97.00 ±1.53	165.00 ± 1.52	230.00 ±1.76	300.00 ± 2.25	285.00 ± 2.52
CD	2.28	3.60	3.81	4.87	3.32

* Mean of three replications. Data after ± represent standard error values.
Dry weight of inoculum was 10 mg.

The fungus showed maximum growth in PDB (490.0 mg) after 20 d of incubation. Growth was rapid during the first 5 d after which the rate of growth decreased and continued until 20 d after which mycelial weight declined. Similar growth pattern was observed in case of OMB and RM.

4.3.3. Mycelial growth of *C. gloeosporioides* at different pH

Sterilized PDB medium (25 ml in 100 ml Erlenmeyer flask) was adjusted to pH 4.0, pH 5.0, pH 5.5, pH 6.0, pH 6.5, pH 7.0 and pH 8.0 seperately. Three flasks were taken for each value. The pH of the medium was adjusted by adding 1 (N) NaOH or 1 (N) HCl drop-wise into the medium before sterilization. The test fungus *C. gloeosporioides* was inoculated separately in the media of different pH according

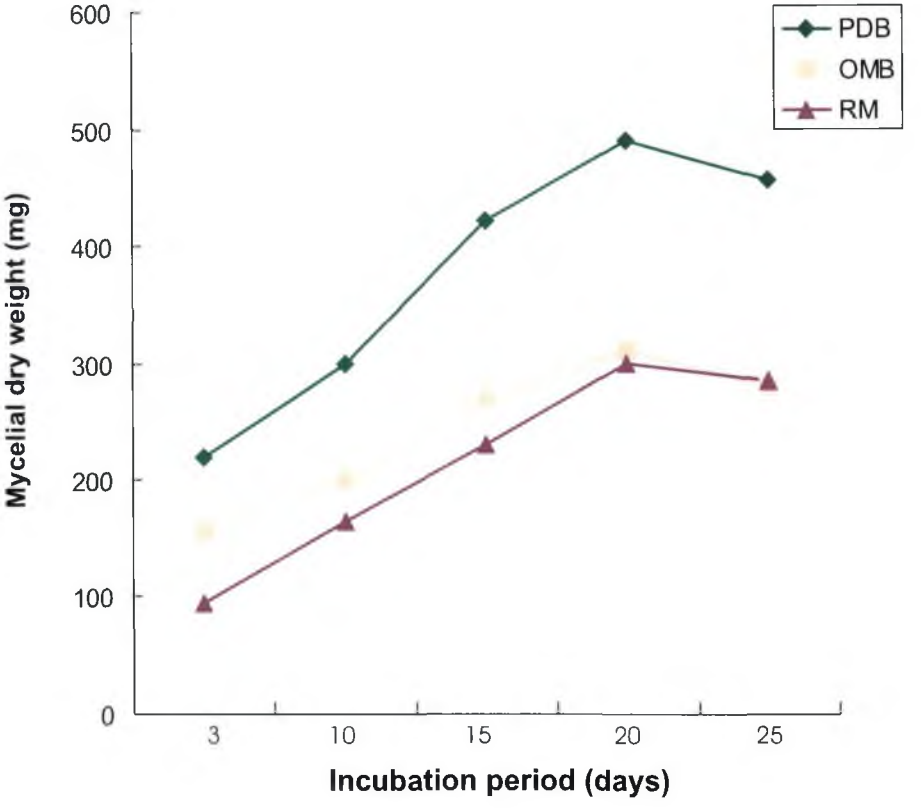

Fig. 7 : Growth of *Colletotrichum gloeosporioides* after different incubation periods in three different liquid media.

Abbreviations : PDB = Potato dextrose broth
OMB = Oat meal broth
RM = Richard's medium

to the method described under materials and methods in section 3.8.2. and incubated at 28±1 °C. Mycelial dry weights after 3, 6, 9, 12 and 15 d of incubation were noted and tabulated in Table 7 and Fig. 8.

Table 7. Effect of different pH on growth of *Colletotrichum gloeosporioides* in PDB.

pH	Mycelial dry weight (mg)* after incubation period (Days)				
	3	6	9	12	15
4.0	35.00 ± 1.73	135.00 ± 1.53	180.00 ± 1.53	165.00 ± 1.53	150.00 ± 1.15
5.0	27.00 ± 1.15	71.00 ± 1.15	152.00 ± 1.53	170.00 ± 1.15	148.00 ± 1.53
5.5	38.00 ± 1.15	150.00 ± 1.17	190.00 ± 1.73	225.00 ± 2.08	210.00 ± 1.73
6.0	40.00 ± 1.00	155.00 ± 2.00	200.00 ± 2.52	231.00 ± 1.54	215.00 ± 1.00
6.5	32.00 ± 1.15	142.00 ± 1.73	188.00 ± 2.08	215.00 ± 1.53	200.00 ± 1.53
7.0	34.00 ± 1.00	142.00 ± 1.53	185.00 ± 2.08	175.00 ± 1.53	160.00 ± 2.08
8.0	10.00 ± 1.00	50.00 ± 1.53	75.00 ± 1.53	105.00 ± 2.08	115.00 ± 1.15
CD at 5%	2.30	3.44	4.00	3.02	3.09

* Mean of three replications. Data after ± represent standard error values.
Dry weight of inoculating mycelial block was 10 mg.

Mycelial dry weight of *C. gloeosporioides* was maximum (231.0 mg) at pH 6.0 and minimum at pH 8.0 (105.0 mg) after 12 d of inoculation. For each pH, the mycelial dry weights were maximum after 12 d of inoculation except pH 4.0 and pH 8.0. At pH 4.0, the maximum mycelial dry weight was recorded after 9 d of inoculation while in case of pH 8.0, the maximum mycelial dry weight was recorded after 15 d of inoculation.

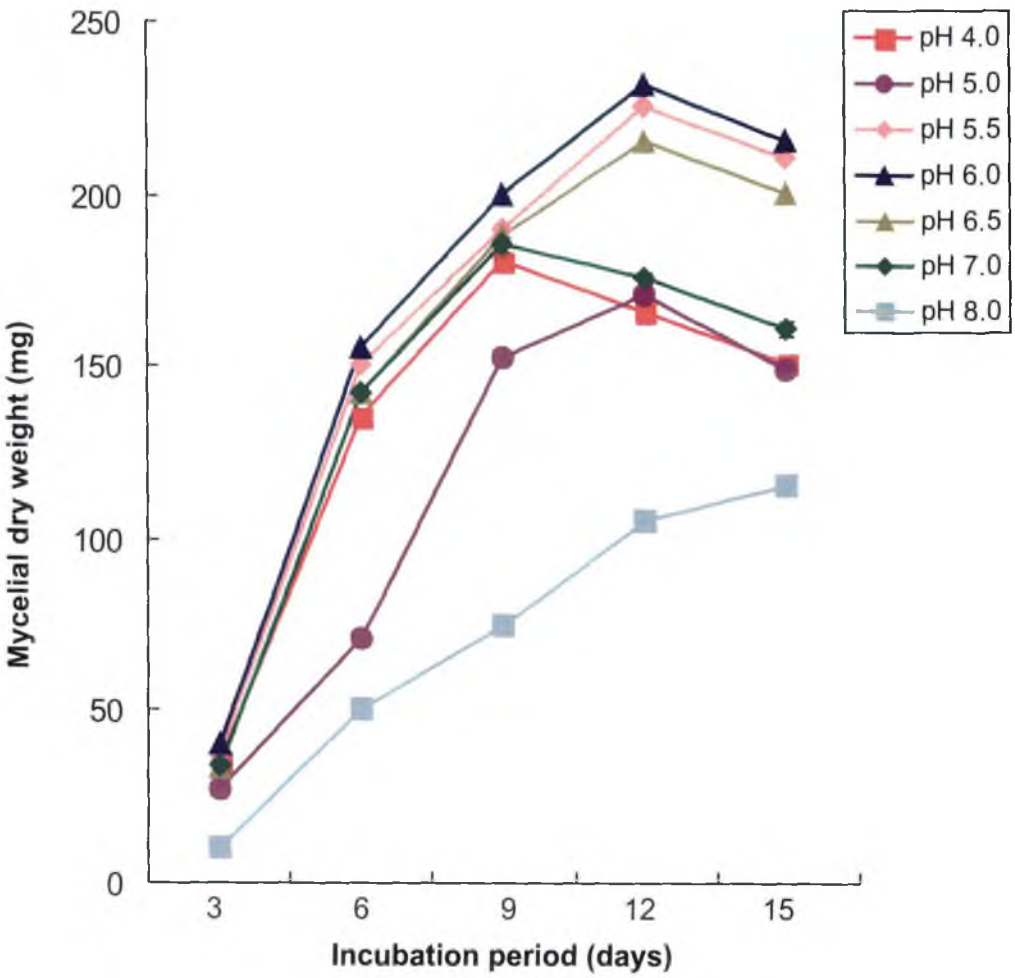

Fig. 8 : Growth of *Colletotrichum gloeosporioides* under different pH conditions.

4.3.4. Mycelial growth of *C. gloeosporioides* at different temperature

The growth of *C. gloeosporioides* was assessed at different temperatures. The test fungus was inoculated in sterile PDB (25 ml in 100 ml Erlenmeyer flask) according to the method described in section 3.8.2. The inoculated flasks were incubated at different temperatures viz. 8 °C, 13 °C, 18 °C, 23 °C, 28 °C, 33 °C and 38 °C in different sets of triplicates. After 3, 6, 9, 12 and 15 d of incubation, the fungal mycelia were harvested and strained through muslin cloth, blotted and dried at 60 °C. Finally the dried mycelia were cooled and mycelial dry weights were noted and presented in Table 8 and Fig. 9.

Table 8. Effect of different temperatures on mycelial growth of *Colletotrichum gloeosporioides* in PDB.

Temperatures (°C)	Mycelial dry weight (mg)* after incubation period (Days)				
	3	6	9	12	15
08	10.00 ± 1.04	10.00 ± 0.50	10.00 ± 1.00	10.00 ± 0.50	10.00 ± 0.58
13	13.00 ± 1.00	18.00 ± 1.00	55.00 ± 1.00	60.00 ± 1.00	60.00 ± 3.51
18	22.00 ± 1.26	58.00 ± 2.08	125.00 ± 1.73	158.00 ± 1.53	145.00 ± 1.53
23	24.00 ± 0.76	135.00 ± 2.00	182.00 ± 3.05	208.00 ± 2.00	195.00 ± 1.73
28	25.00 ± 1.53	145.00 ± 2.08	198.00 ± 2.52	225.00 ± 3.21	210.00 ± 1.53
33	23.00 ± 1.26	125.00 ± 0.58	176.00 ± 2.08	195.00 ± 1.00	181.00 ± 2.65
38	15.00 ± 1.26	31.00 ± 1.15	46.00 ± 2.00	55.00 ± 1.53	50.00 ± 2.08
CD at 5%	2.37	3.08	4.30	3.24	4.20

* Mean of three replications. Data after ± represent standard error values.
Dry weight of inoculating mycelial block was 10.0 mg.

Results revealed that maximum growth was possible at 28 °C at which the mycelial dry weight was recorded as 225 mg after 12 d of inoculation. Growth was usually maximum after 12 d of inoculation after which it declined. No growth was recorded at 8 °C even after 15 d of inoculation.

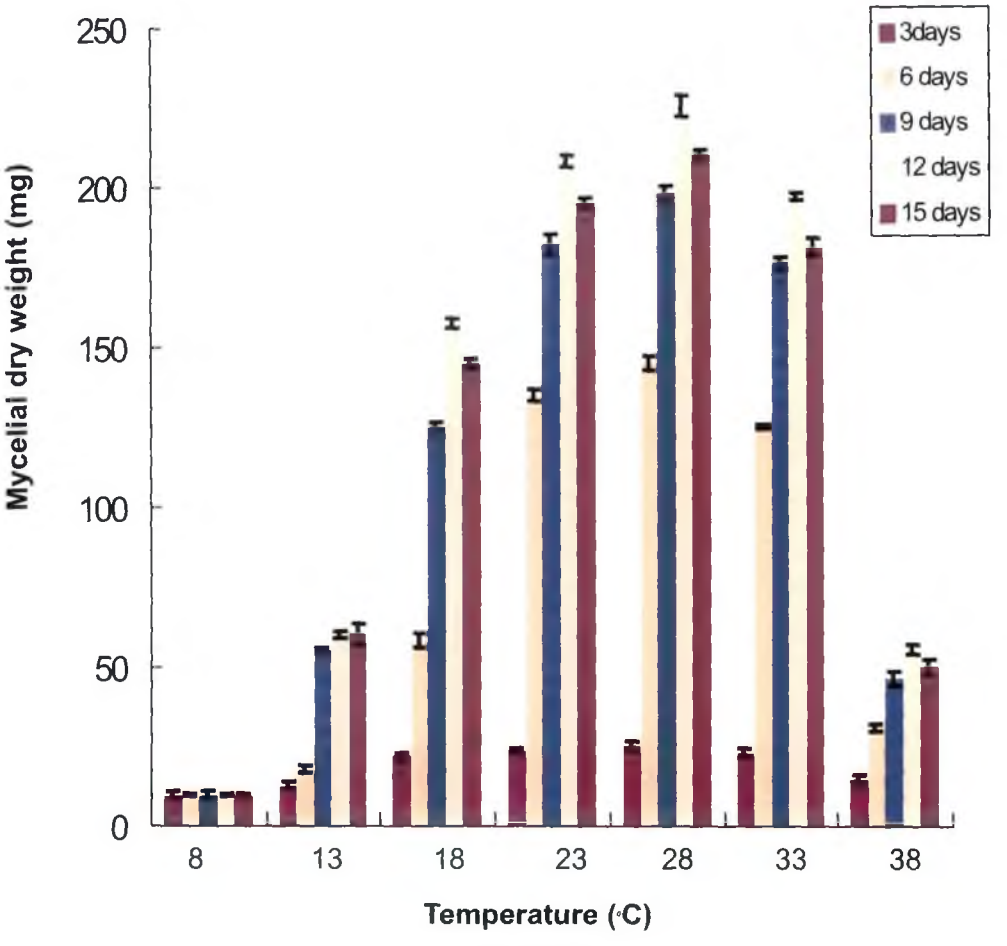

Fig. 9 : Graphical representation of effect of different temperature on mycelial growth of *Colletotrichum gloeosporioides*.

4.3.5. Assessment of mycelial growth of *C. gloeosporioides* on different carbon sources

The nutritional requirements of a pathogen are of considerable interest as it may help in understanding the physiology of disease development. Although there are some reports on nutritional physiology of *C. gloeosporioides*, it was considered worthwhile to study different carbon sources for the optimum growth and sporulation of the isolated strain in the present study. A basal medium (Glucose 1%; Asparagine 0.2%; KH_2PO_4 0.1%; $MgSO_4$, $7H_2O$ 0.05%; Zn^{++}, $Mn^{++}$and Fe^{+++} 2 µg/ml) was used for the purpose. The different carbon sources tested were glucose, sorbitol, sucrose, lactose, mannitol and inositol. The equivalent amount of carbon present in 1% glucose was used as standard and added separately to the basal medium. The medium (25 ml) was taken in 100 ml Erlenmayer flasks and sterilized by autoclaving at 15 lb. p.s.i. for 15 minutes. Three flasks were taken for each carbon source. After cooling, the pathogen was inoculated using 4 mm mycelial discs in PDA and incubation was allowed for 3, 6, 9,12 and 15 days. Erlenmeyer flasks of 100 ml capacity were used where each flask contained 25 ml of sterilized media. In control sets, no carbon sources were used in the basal medium. After incubation for the specified time periods, the mycelia were harvested, dried at 60 °C and weighed. The results were tabulated in Table 9. After each incubation period, the extracts of sporulation was also recorded in five different grades on the basis of visual observations.

From the results of Table 9 and Fig. 10, it was evident that *C. gloeosporioides* showed a gradual increase in growth until 12 d in all cases except in lactose as carbon source. In the control sets, gradual increase in growth until 15 d of incubation was observed. Mycelial dry weight was maximum (105 mg) after 12 d of incubation when mannitol was used as carbon source. Sorbitol supplied media produced mycelial growth (100 mg) next to mannitol, after 12 d of incubation. When lactose was used as carbon source, the growth was minimum among the carbon sources tested and it was only 49 mg after 15 d of incubation. Mannitol was found as the best carbon source among the different carbon sources tested when overall growth pattern of *C. gloeosporioides* was observed. Sporulation was excellent in mannitol and sorbitol after 12 d of incubation. In all other cases, sporulation was graded as good, fair, poor and nil. Generally good sporulation was found after 12 d of incubation. In case of glucose, inositol and sucrose, good

sporulation was observed after 15 d of incubation. After 6 d of incubation, sporulation started in all the cases except lactose. In control set, insignificant growth was observed without any sporulation.

Table 9. Effect of different carbon sources on the growth and sporulation of *Colletotrichum gloeosporioides*.

Carbon source	Incubation period (Days)									
	3		6		9		12		15	
	Mwt* (mg)	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Glucose	10.00 ±0.50	–	21.00 ±1.53	++	71.00 ±2.52	+++	86.00 ±2.00	+++	80.00 ±1.00	++++
Sorbitol	11.00 ±0.50	–	24.00 ±0.50	+++	82.00 ±1.15	+++	100.00 ±2.52	++++	90.00 ±2.02	++++
Sucrose	18.00 ±1.00	–	28.00 ±1.53	++	50.00 ±1.32	++	56.00 ±0.76	++	55.00 ±0.58	+++
Lactose	10.00 ±1.00	–	19.00 ±1.15	_	30.00 ±1.50	+	45.00 ±0.58	++	40.00 ±1.80	++
Mannitol	12.00 ±1.00	–	23.00 ±0.76	++	86.00 ±1.04	+++	105.00 ±1.53	++++	96.00 ±1.50	++++
Inositol	10.00 ±0.57	–	21.00 ±1.00	+	69.00 ±1.44	++	92.00 ±2.52	+++	81.00 ±2.00	++++
Control***	4.00 ±0.50	–	8.00 ±0.76	–	14.00 ±0.76	–	18.00 ±1.53	–	20.00 ±0.76	–
CD at 5%	1.56		2.28		3.04		3.41		3.00	

Mean of 3 replications. Data after ± represent standard error values.
*Mwt (mg) = Mycelial dry weight in mg.
** Spn = sporulation, – = Nil, + = Poor, ++ = Fair, +++ = Good, ++++ = Excellent.
*** Basal medium without any carbon source.

4.3.6. Assessment of mycelial growth of *C. gloeosporioides* on different nitrogen sources

To assess mycelial growth and sporulation of *C. gloeosporioides* on different nitrogen sources (both inorganic and organic). Modified Asthana and Hawker's medium 'A' (glucose 10 g; KNO_3 3.5 g; KH_2PO_4 1.75 g; $MgSO_4$, $7H_2O$ 0.75 g; agar 20 g and distilled water 1L) without agar was used as basal medium. The quantity of

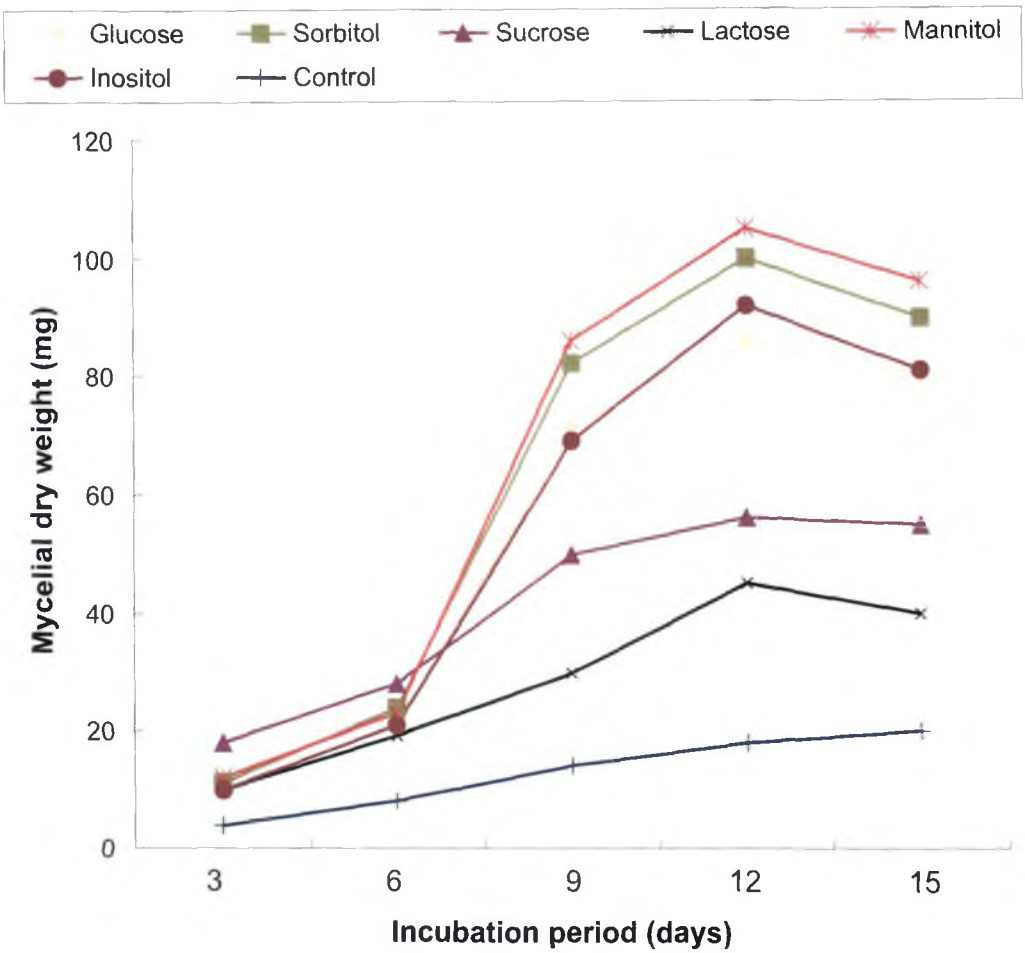

Fig. 10 : Graphical representation of effect of different carbon sources on mycelial growth of *Colletotrichum gloeosporioides*.

various nitrogen sources was so adjusted as to give the same amount of nitrogen as furnished by 3.5 g of KNO_3 in the basal medium. The medium with required nitrogen sources was prepared and 25 ml of media was dispensed in each 100 ml Erlenmeyer flask taking 3 flasks for each Nitrogen source. All the media were sterilized by autoclaving at 15 lb. p.s.i. for 15 minutes and after cooling, the test fungus was inoculated and incubated for 3, 6, 9, 12 and 15 days at 28±1°C temperature. In control set, no nitrogen source was used in the basal medium. After specified incubation periods, the mycelia were harvested, dried at 60 °C and weighed. The results are tabulated in Table 10. After each of the different incubation periods, the extent of sporulation was also recorded in five different grades based on visual observations.

From the results (Table 10 and Fig. 11) it was evident that *C. gloeosporioides* showed highest growth in peptone (82.00 mg) after 12 d of incubation. The other two organic nitrogen sources, beef extract and yeast extract also showed satisfactory growth (63.00 mg and 60.33 mg respectively) after 12 d of incubation. Among the inorganic nitrogen sources tested, $(NH_4)_2SO_4$ and $(NH_4)_3PO_4$ showed best results (57.17 mg and 42.00 mg respectively) after 12 d of incubation. Media containing potassium nitrate as nitrogen source showed minimum (15 mg after 12 d) mycelial growth among all the sources tested. In all cases, maximum increase in growth was observed within first 6 d of incubation and it declined after 12 d. Sporulation was found good after 12 d of incubation and continued until 15 d. In case of peptone and yeast extract, sporulation was found excellent after 15 d of incubation. Good sporulation was also observed in beef extract as nitrogen source after 15 d of incubation. Insignificant growth without any sporulation was observed in control set.

4.3.7. Conditions affecting sporulation of *C. gloeosporioides*

Physical conditions required for spore germination are very important for understanding the host-pathogen interaction. For this, different incubation period, different pH and different temperatures are very important in determining the germination, appressoria formation as well as germ tube elongation. Like many other physiological activities, light has some role in germination and germ tube elongation. During the present study, germination, germ tube elongation and appressoria formation was slightly more in dark than when incubated in normal light at 28±1 °C (data not shown).

Table 10. Effect of different nitrogen sources on the growth and sporulation of *Colletotrichum gloeosporioides.*

Nitrogen sources	Incubation period (Days)									
	3		6		9		12		15	
	Mwt* (mg)	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Organic										
Peptone	17.50 ±0.29	–	44.50 ±0.86	+	70.67 ±1.20	++	82.00 ±2.65	+++	70.17 ±1.96	++++
Yeast extract	36.33 ±1.76	–	52.23 ±1.13	+	60.00 ±1.00	+	60.33 ±2.52	+++	60.17 ±1.48	++++
Beef extract	30.17 ±1.09	–	46.17 ±1.09	+	57.00 ±1.15	+	63.00 ±2.65	++	55.67 ±1.45	+++
Glycine	11.67 ±0.67	–	15.17 ±0.83	–	16.83 ±1.09	+	15.17 ±1.04	++	14.83 ±0.60	++
Trypton	29.50 ±1.26	–	44.00 ±1.53	–	51.17 ±1.92	+	56.17 ±1.89	++	51.33 ±1.20	++
Inorganic										
Ammonium sulphate	5.67 ±0.17	–	20.00 ±0.50	–	46.17 ±0.60	–	57.17 ±1.61	–	50.00 ±1.04	–
Ammonium phosphate	5.33 ±0.17	–	14.17 ±0.44	–	32.00 ±1.00	–	42.00 ±1.73	–	42.00 ±1.53	–
Ammonium nitrate	5.17 ±0.60	–	13.33 ±0.67	–	25.33 ±0.88	–	35.33 ±1.53	–	30.17 ±0.93	–
Sodium nitrate	4.67 ±0.44	–	5.83 ±0.33	–	10.17 ±0.60	–	15.83 ±1.04	–	15.67 ±0.67	–
Potassium nitrate	4.17 ±0.33	–	5.17 ±0.60	–	10.33 ±0.33	–	15.00 ±1.73	–	12.00 ±0.50	–
Control***	4.33 ±0.44	–	5.17 ±0.33	–	6.17 ±0.33	–	7.83 ±0.76	–	8.67 ±0.33	–
CD at 5%	1.30		1.30		1.64		1.70		1.88	

Mean of 3 replications. Data after ± represent standard error values.

*Mwt (mg) = Mycelial dry weight in mg.

** Spn = sporulation, – = Nil, + = Poor, ++ = Fair, +++ = Good, ++++ = Excellent.

*** Basal medium without any carbon source.

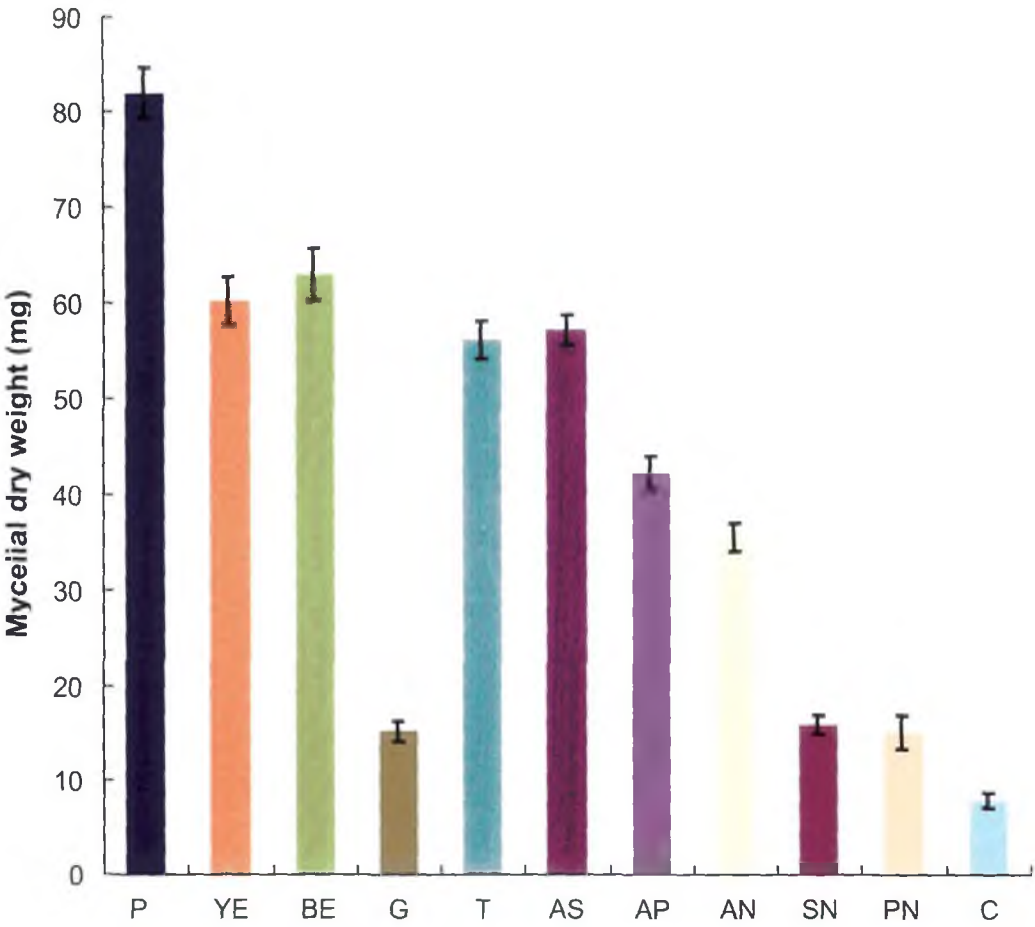

Fig. 11 : Graphical representation of effect of different nitrogen sources on mycelial growth of *Colletotrichum gloeosporioides* after 12 d of incubation

Abbreviations: P = Peptone
YE = Yeast extract
BE = Beef extract
G = Glycine
T = Trypton
AS = Ammonium sulphate
AP = Ammonium phosphate
AN = Ammonium nitrate
SN = Sodium nitrate
PN = Potassium nitrate
C = Control

4.3.7.1. Spore germination, germ tube elongation and appressoria formation of *C. gloeosporioides* after different periods of incubation

Spore germination, germ tube elongation and appressoria formation of *C. gloeosporioides* was studied after different periods of incubation in two different ways. Spore suspensions ($1x10^6$ ml^{-1}) prepared with sterile distilled water was used in one set. Spore suspensions (30 µl) was placed on microscopic slides in triplicates and allowed to incubate for 6, 12, 18, 24, 30, 36, 42 and 48 hours at 28±1 °C in a humid chamber as described under materials and methods (Section 3.8.3.2). In another set, spore suspension was prepared in low concentration (1:16) of brinjal leaf extract (1 g/ 10 ml distilled water) in sterile distilled water. This spore suspension (30 µl) was similarly placed on microscopic slides in triplicates and allowed to incubate for 1, 2, 3, 4, 5, 6, 7 and 8 hours at 28±1 °C in a humid chamber. The results were tabulated in Table 11 and Table 12.

Table 11. Effect of different incubation periods on spore germination, germ tube elongation and appressoria formation of *Colletotrichum gloeosporioides*

Incubation period (hours)	Percent[1] germination	Germ tube[2] length (µm)	Percent appressoria[3] formation
6	0.00±0.00	0	0
12	9.83±0.67	14.33±0.60	0
18	22.17±1.59	26.67±0.88	6.83±0.44
24	43.67±2.03	40.50±1.76	23.50±1.32
30	53.00±1.15	62.00±2.65	38.00±1.00
36	69.33±1.45	75.83±1.48	52.50±1.26
42	78.00±2.08	92.33±2.40	58.83±1.48
48	87.17±1.07	97.33±2.35	64.67±1.59
CD at 5%	1.81	2.42	1.26

[1] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
[2] Mean of 3 replications and average of 60 germ tubes per slide.
[3] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
Data after ± represent standard error values.

Germination of spores started after 10-12 hours of incubation when sterile distilled water was used in spore suspension. An increase in the percent germination of spores, germ tube length and percent appressoria formed were observed with increasing time interval and after 48 h, these were recorded as 87.17%, 97.33 µm and 64.67% respectively (Table 11 and Fig. 12).

Table 12. Effect of different incubation periods on spore germination, germ tube elongation and appressoria formation of *C. gloeosporioides* adding brinjal leaf extract.

Incubation period (hours)	Percent[1] germination	Germ tube length[2] (µm)	Percent[3] appressoria formation
1	0	0	0
2	5.43±0.29	7.83±0.27	0
3	49.43±1.53	14.76±0.45	0
4	82.67±0.93	25.37±0.63	0
5	89.83±1.36	44.43±0.79	6.73±0.37
6	93.50±0.86	70.73±1.18	19.43±1.03
7	96.60±1.05	103.33±2.19	38.6±1.45
8	98.30±0.40	142.50±2.29	62.50±1.44
CD at 5%	1.85	1.76	1.39

[1] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
[2] Mean of 3 replications and average of 60 germ tubes per slide.
[3] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
Data after ± represent standard error values.

When brinjal leaf extract was added in spore suspension, germination of spores started after 2 hours of incubation and nearly all the spores were germinated within 8 h of incubation (Table 12 and Fig. 13). The percent germination of spores, germ tube length and percent appressoria formed were 98.30%, 142.50 µm and 62.50% respectively after 8 h of incubation.

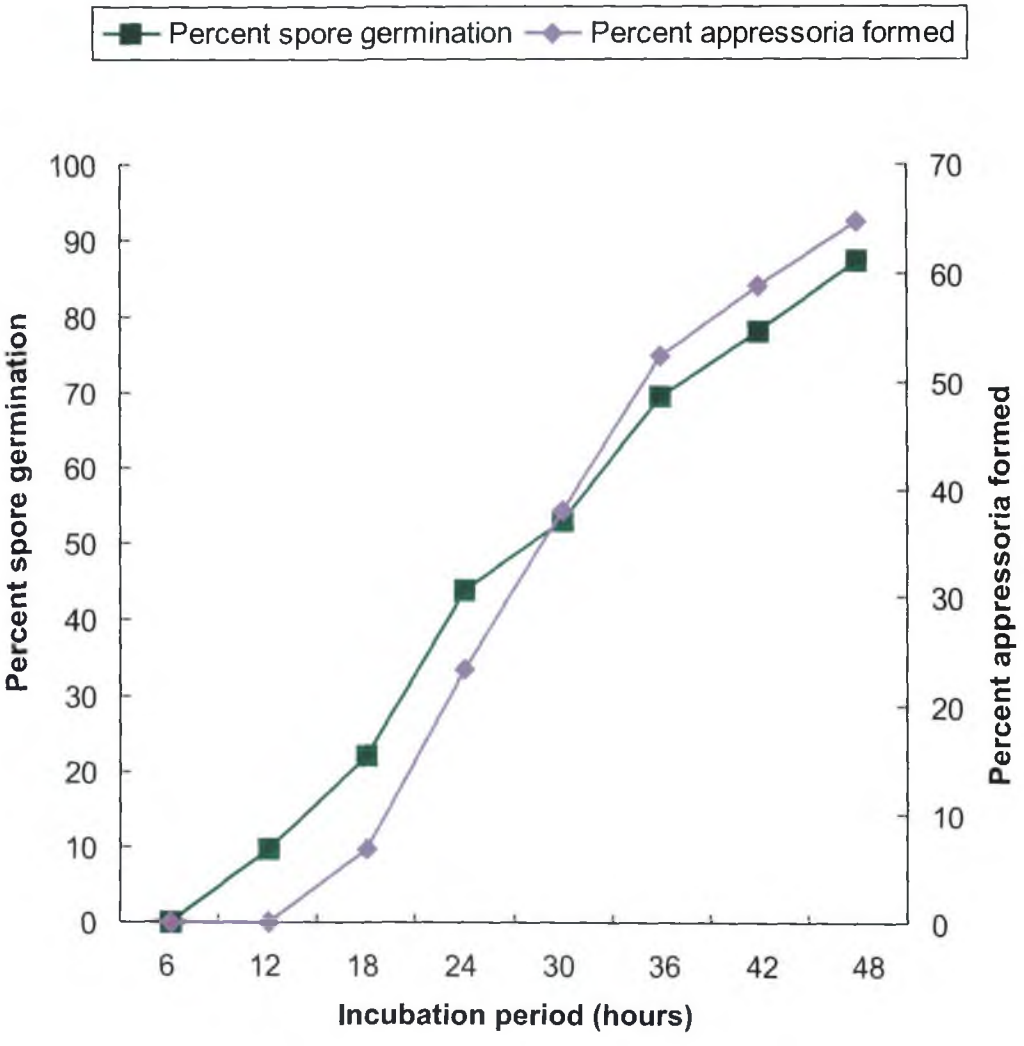

Fig. 12 : Graphical representation of effect of different incubation periods on spore germination and appressoria formation of *Colletotrichum gloeosporioides.*

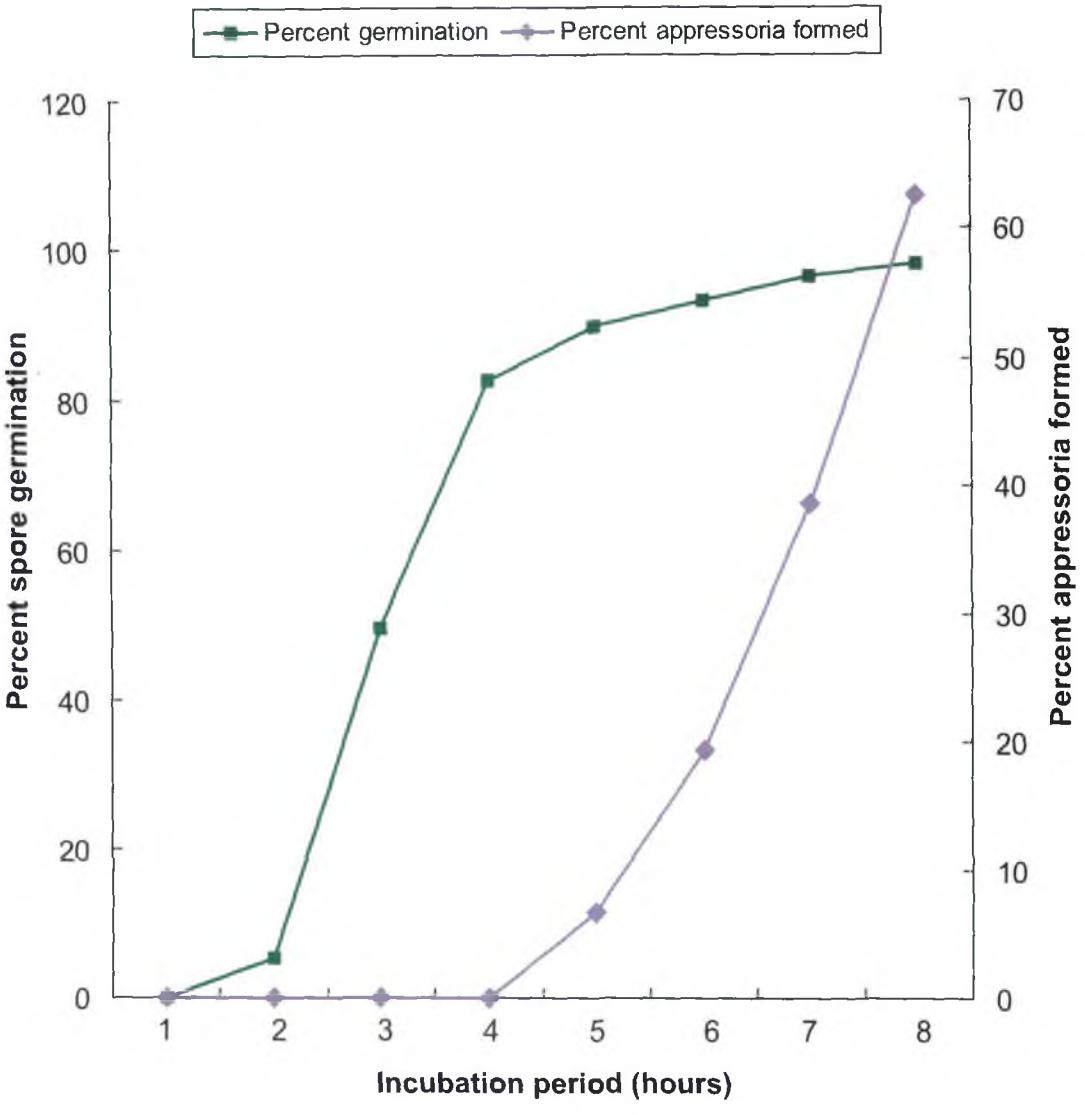

Fig. 13 : Graphical representation of effect of different incubation periods on spore germination and appressoria formation of *Colletotrichum gloeosporioides* adding brinjal leaf extract.

4.3.7.2. Spore germination and germ tube elongation of *C. gloeosporioides* at different pH

Solutions of different pH values of 4.0, 6.0, 6.50, 6.75, 7.25 and 9.0 were prepared by mixing 0.01 M K_2HPO_4 and KH_2PO_4 in required ratios and sterilized. The spores of the test fungus was suspended in the sterile solutions of different pH ($1x10^6$ ml^{-1}) and allowed to germinate on glass slides in triplicates for 48 hours at 28±1 °C. Slide germination technique as mentioned in the materials and methods (Section 3.18.2) was followed.

Among the different pH tested, pH 6.0 showed highest germination percentage and it was recorded as 92.00% (Table 13 and Fig. 14). From the result it was evident that percent germination sharply declined at pH 7.25 and no germination was found at pH 9.00. Highest percent of appressoria formation (65.67%) were observed at pH 6.0 although germ tube growth was comparatively smaller (length 77.17 µm). Germ tube length (142.33 µm) was highest at pH 6.75 where no appressoria were formed and percent germination was only 34.77. Significant reduction in percent germination (21.70%) was observed at pH 7.2 although germ tube length was 132.33 µm.

Table 13. Effect of different pH on spore germination germ tube elongation and appressoria formation of *C. gloeosporioides* after 48 h of incubation.

pH	Percent[1] germination when control raised to 100	Germ tube[2] length (µm)	Percent[3] appressoria formation
4.00	77.83±0.93	31.03 ±0.76	59.23±0.76
6.00	92.00±1.53	77.17±1.59	65.67±1.17
6.50	46.63±1.35	120.67±2.85	2.23±0.20
6.75	34.77±0.89	142.33±2.19	0
7.25	21.70±1.11	132.33±1.76	0
9.00	0	0	0
CD at 5%	1.26	2.31	1.16

[1] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
[2] Mean of 3 replications and average of 60 germ tubes per slide.
[3] Mean of 3 replications. Calculated on the basis of 300 spores per slide.
Data after ± represent standard error values.

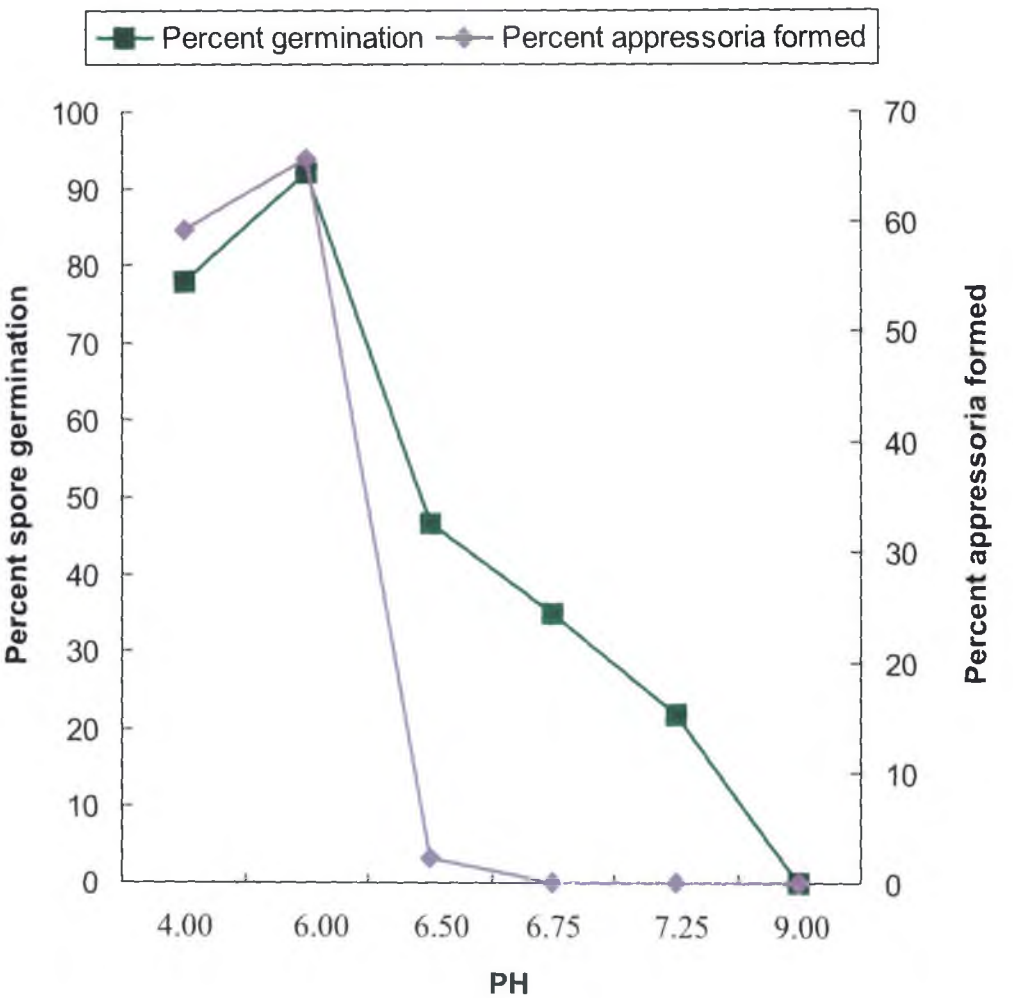

Fig. 14 : Graphical representation of effect of different pH on spore germination and appressoria formation of *Colletotrichum gloeosporioides*.

4.3.7.3. Spore germination and germ tube elongation of *C. gloeosporioides* at different temperature

Spore suspension of *C. gloeosporioides* was prepared as mentioned in the materials and methods (Section 3.8.3.1). Sterile distilled water was added to attain optimum concentration ($1x10^6$ ml^{-1}) of the spores. Spore suspension drops (30 µl) were placed in different slides in triplicates and incubated at different temperatures (10 °C, 15 °C, 20 °C, 25 °C, 28 °C, 30 °C, 35 °C, 40 °C and 45 °C). The results were noted in Table 14.

Table 14. Effect of different temperature on spore germination of *Colletotrichum gloeosporioides* at different incubation period.

Temperature (°C)	Germination (%) after hours*					
	6	12	18	24	36	48
10	0	0	0	0	0	0
15	0	7.23 ±0.64	13.07 ±0.83	24.57 ±1.69	44.37 ±1.93	60.50 ±2.02
20	0	8.77 ±0.72	19.67 ±1.01	36.50 ±1.04	56.23 ±1.86.41	70.50 ±2.78
25	0	9.83 ±1.09	21.67 ±1.01	42.00 ±1.44	70.33 ±1.01	88.00 ±1.26
28	0	11.60 ±0.69	24.50 ±1.26	47.50 ±1.26	72.83 ±2.59	93.53 ±0.78
30	0	8.60 ±0.70	14.73 ±0.90	22.33 ±1.09	41.80 ±1.00	46.23 ±1.11
35	0	2.87 ±0.26	5.77 ±0.54	12.47 ±0.52	17.53 ±1.76	26.50 ±0.85
40	0	0	0	0	0	0
45	0	0	0	0	0	0
CD at 5%		1.01	1.20	1.69	1.88	2.28

*Mean of 3 replications. Percentage calculated on the basis of 300 spores.
Data after ± represent standard error values.

No germination took place within 6 hours of incubation in any of the slides. Spore germination was optimum at 28 °C (93.53% after 48 h). No germination took place at 10 °C or below and 40 °C or above (Table 14 and Fig. 15).

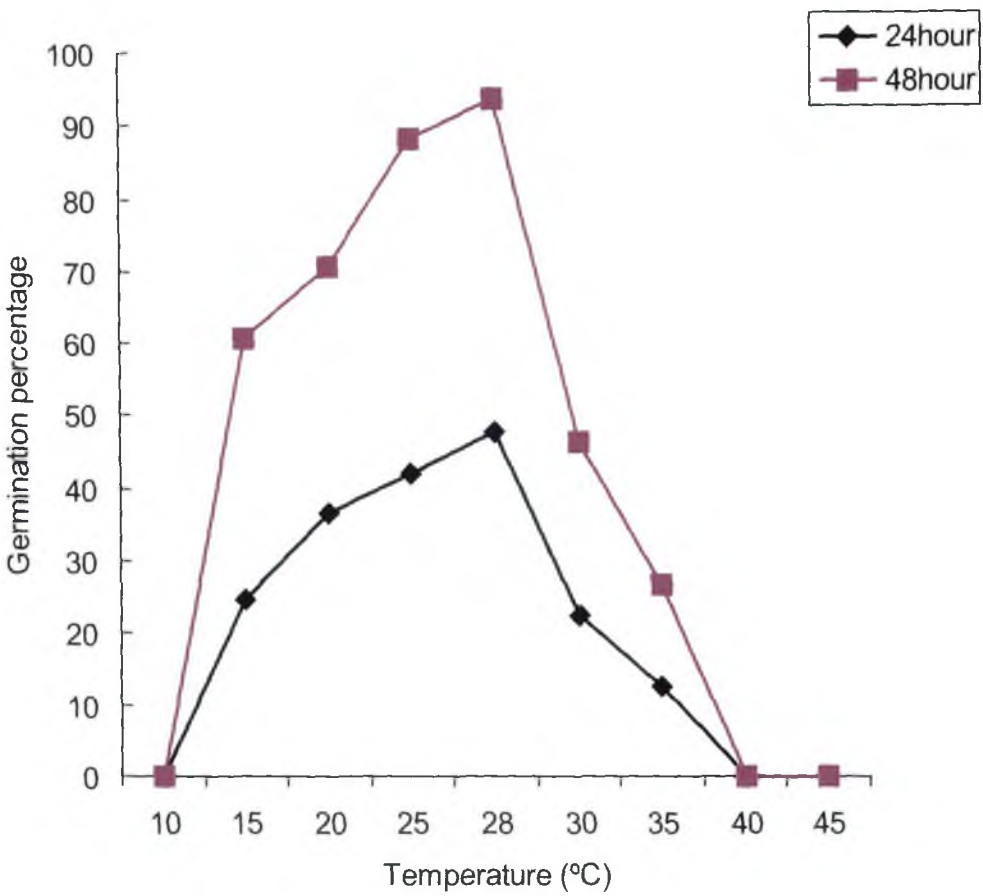

Fig. 15 : Graphical representation of effect of different temperature on spore germination of *Colletotrichum gloeosporioides* at different incubation period.

4.4. Experiment on serological studies

Serology is a powerful tool in virulence studies which determines on one hand, the pathogenic properties of isolates, and on the other hand, the susceptibility or resistance of host cultivars. In the present investigation, attempt has been taken to determine the presence of common antigens, if any, among the brinjal varieties and *C. gloeosporioides*. Plant antigens were prepared from healthy leaves of 28 brinjal varieties (including susceptible and resistant cultivated varieties). Similarly fungal antigens were prepared from a virulent isolate of *C. gloeosporioides*. Polyclonal antisera were raised in separate male white rabbits against antigens of selected representative type i.e. one resistant (Shamala variety) and one susceptible (Pusa purple long) brinjal varieties and pathogen (*C. gloeosporioides*). Normal sera were collected from rabbits by marginal ear vein puncture before immunization.

4.4.1. Relationship between different brinjal varieties and *C. gloeosporioides* by agar gel double diffusion

The standard method as described by Ouchterlony (1958) was followed to determine the serological relationship between host and pathogens by agar gel double diffusion. Semi quantitative estimation of antibody activity of the above mentioned three different antisera against their homologous antigens as well as titre values of antigens of Pusa purple long, Shamala varietiy and *C. gloeosporioides* against their homologous antisera were determined. Results are presented in Table 15. The antigen of the pathogen *C. gloeosporioides*, antigen of non-pathogen *Alternaria porri*, leaf antigens of susceptible brinjal varieties (Pusa purple long, Pusa purple round, Pant brinjal 4, BE-706, Orissa green and Green round) as well as leaf antigens of resistant varieties (Shamala variety, Lalguli variety , Preeti variety, Aam begun and Kuroi variety) were used in the experiment. The antisera used in the experiments were antisera of *C. gloeosporioides* (CgA), antisera of leaves of Pusa purple long (PplA) and Shamala variety (ShavA). Preparation of the antigens, antisera and details of the procedure has already been discussed in the materials and methods section (Section 3.13.1). The results are shown in Table 16.

Table 15. Semi quantitative estimation of antigens and antisera of brinjal varieties and *C. gloeosporioides.*

Host and pathogen	Titre of antigen against homologous antiserum	Titre of antiserum against homologous antigen
Host variety		
Shamala	8	16
Pusa purple long	8	16
Pathogen		
C. gloeosporioides	16	32

Incubation time- 72h
Temperature- 25±1° C.

Table 16. Common antigenic relationship between brinjal varieties and *Colletotrichum gloeosporioides* (Based on agar gel double diffusion)

Antigen of pathogen, host and non-pathogen	Antisera of pathogen and host		
	C. gloeosporioides* (CgA)	**Pusa purple long (PplA)**	**Shamala (ShavA)**
Pathogen			
C. gloeosporioides	+	+	–
Susceptible varieties			
Pusa purple long (Ppla)	+	+	+
Pusa purple round (Ppra)	+	+	+
Pant brinjal 4 (Pb4a)	+	+	+
BE-706 (B706a)	+	+	+
Orissa green (Oga)	+	+	+
Green round (Gra)	+	+	+
Resistant varieties			
Shamala variety (Shava)	–	+	+
Lalguli variety (Lva)	–	+	+
Preeti variety (Pva)	–	+	+
Kuroi variety (Kva)	–	+	+
Aam begun (Aba)	–	+	+
None pathogen			
Alternaria porri (Apa)	–	–	–

Common precipitation band present = +
Common precipitation band absent = –
*Codes of different antigens and antisera are in parenthesis.

From the results noted in Table 16, it was observed that common antigenic relationship were present not only in cases of homologous reactions i.e. between antisera and antigens of *C. gloeosporioides* (Plate IX : B & D), Pusa purple long (Plate IX : A & E) and Shamala variety (Plate X : B) but also in cross reactions between antisera of *C. gloeosporioides* and antigens from leaves of Pusa purple long (Plate IX : D), Pusa purple round (Plate IX : D), Pant brinjal 4 (Plate IX : D), Orissa green (Plate IX : D), Green round (Plate IX : D). No precipitation bands were observed when antigens of Shamala variety, Lalguli variety , Preeti variety and Kuroi variety (Plate IX : B) were used against antisera of *C. gloeosporioides*. Common precipitation bands were also found in reactions between antisera of Pusa purple long and antigens of *C. gloeosporioides* (Plate IX: E & F), Pusa purple round (Plate IX : F), Pant Brinjal 4 (Plate IX: A & X : A), Orissa green (Plate IX: A & X : A) and Shamala variety & Lalguli variety (Plate X : A). Where the antisera of Shamala variety were used, common precipitation bands were found with the antigens of Pusa purple long, Pusa purple round, Pant brinjal 4, BE-706, Orissa green, Green round, Shamala variety, Lalguli variety, Preeti variety and Kuroi variety. No precipitation bands were observed in any reaction involving antigen of *A. porri*.

Immunodiffusion tests clearly showed the presence or absence of common antigens between hosts and pathogen. Presence of common antigen was represented due to single or several antigenic substances was not clear. Many of the antigen antiserum reactions (precipitin bands) could not be clearly distinguished. Hence for better resolution it was decided to seperate the antigens by electrophoresis before exposing them to antisera.

4.4.2. Serological relationship between different brinjal varieties and test pathogen *C. gloeosporioides* by immunoelectrophoresis

A combination of electrophoresis and radial immunodiffusion in agar gel is immunoelectrophoresis. In agar gel, movement of molecules in an electric field is similar to that in liquid medium, with the advantage that free diffusion during and after electrophoresis is lessened (Clausen, 1969).

Following standard method, immunoelectrophoresis was done using the antisera of *C. gloeosporioides,* Pusa purple long and Shamala variety and antigens of *C. gloeosporioides,* different brinjal varieties and non-pathogen *Alternaria porri.*

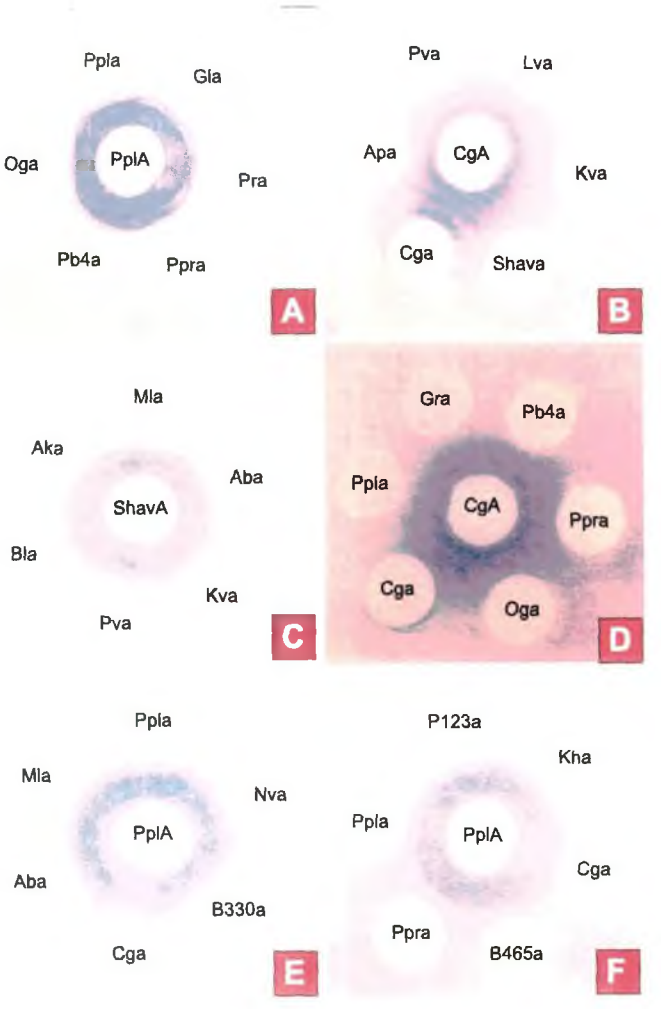

Plate IX : Agar gel double diffusion test using different antigens and antisera.

Fig. A : Leaf antigens of Pusa purple long (Ppla), Green long (Gla), Pant rituraj (Pra), Pusa purple round (Ppra), Pant brinjal-4 (Pb4a) & Orissa green (Oga) were used in peripheral wells while central well contained antisera of Pusa purple long (PplA).

Fig. B : Leaf antigens of Preeti variety (Pva), Lalguli variety (Lva), Kuroi variety (Kva), Shamala variety (Shava), mycelial antigen of *C. gloeosporioides* (Cga) and non-pathogen *Alternaria porri* (Apa) were used in peripheral wells while central well contained antisera of *C. gloeosporioides* (CgA).

Fig. C : Leaf antigens of Muktakeshi local (Mla), Aam begun (Aba), Kuroi variety (Kva), Preeti variety (Pva), Baromasi local (Bla), Arka keshav (Aka) were used in peripheral wells while central well contained antisera of Shamala variety (ShavA).

Fig. D : Leaf antigens of Green round (Gra), Pant brinjal-4 (Pb4a), Pusa purple round (Ppra), Orissa green (Oga) & Pusa purple long (Ppla) and mycelial antigen of *C. gloeosporioides* (Cga) were used in peripheral wells while central well contained antisera of *C. gloeosporioides* (CgA).

Fig. E : Leaf antigens of Pusa purple long (Ppla), Nisha variety (Nva), BSS-330 (B330a), Muktakeshi local (Mla), & Aam begun (Aba) and mycelial antigen of *C. gloeosporioides* (Cga) were used in peripheral wells while central well contained antisera of Pusa purple long (PplA).

Fig. F : Leaf antigens of PK-123 (P123a), Kanha hybrid (Kha), Pusa purple long (Ppla), Pusa purpl round (Ppra), & BSS 465 chhaya (B465a) and mycelial antigen of *C. gloeosporioides* (Cga) were used in peripheral wells while central well contained antisera of Pusa purple long (PplA).

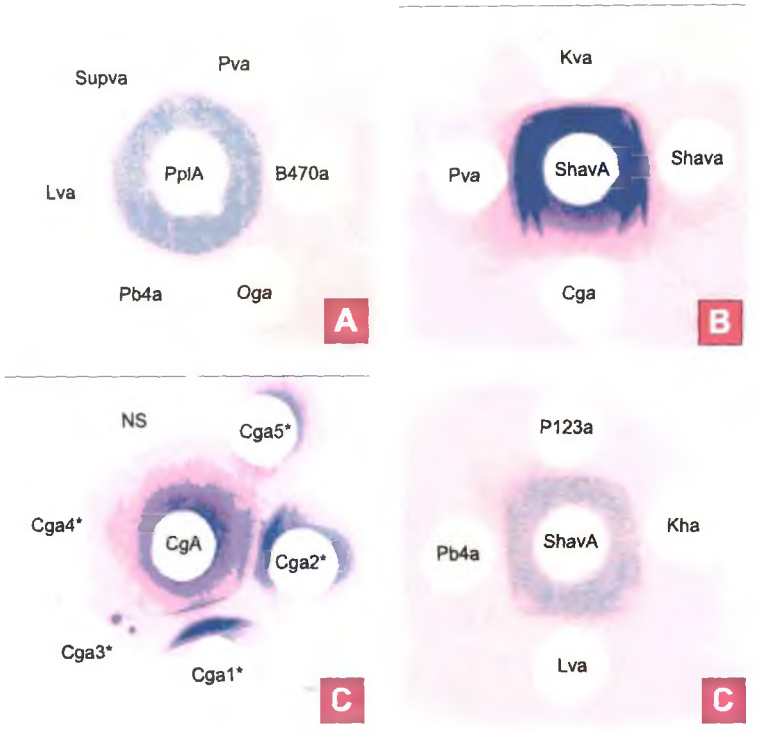

Plate X : Agar gel double diffusion test using different antigens and antisera.

Fig. A : Leaf antigens of Supriya variety (Supva), Preeti variety (Pva), BSS 470 janam (B470a), Orissa green (Oga), Pant brinjal-4 (Pb4a) & Lalguli variety (Lva) were used in peripheral wells while central well contained antisera of Pusa purple long (PplA).

Fig. B : Leaf antigens of Preeti variety (Pva), Kuroi variety (Kva), Shamala variety (Shava) and mycelial antigen of *C. gloeosporioides* (Cga) were used in peripheral wells while central well contained antisera of Shamala variety (ShavA).

Fig. C : Normal sera (NS) and different dilution of mycelial antigen of *C. gloeosporioides* (Cga1*-no dilution, Cga2*-1:1 dil., Cga3*-1:2 dil., Cga4*-1:4 dil.and Cga5*-1:8 dil.) were used in peripheral wells while central well contained antisera of *C. gloeosporioides* (CgA).

Fig. D : Leaf antigens of PK-123 (P123a), Kanha hybrid (Kha), Lalguli variety (Lva) & Pant brinjal-4 (Pb4a) were used in peripheral wells while central well contained antisera of Shamala variety (ShavA).

The detail of the procedure has already been discussed in the materials and method (Section 3.13.2). The results of the experiments are noted in Table 17 and Table 18.

Table17. Comparison of precipitation arcs found in immunoelectrophoresis of brinjal varieties (Susceptible and resistant), pathogen (*C. gloeosporioides*) and non-pathogen (*Alternaria porri*).

Antigen of pathogen, host and non pathogen	Total no of precipitation arcs		
	Antisera of pathogen and host		
	C. gloeosporioides (CgA)*	Pusa purple long (PplA)	Shamala Variety (ShavA)
Pathogen			
C. gloeosporioides	3	1	0
Susceptible varieties			
Pusa purple long (Ppla)	1	4	2
Pusa purple round (Ppra)	1	4	3
Pant brijal-4 (Pb4a)	1	4	2
PK-123 (P123a)	1	4	2
Resistant varieties			
Shamala variety (Shava)	0	2	4
Lalguli variety (Lva)	0	2	2
Preeti variety (Pva)	0	2	3
Kuroi variety (Kva)	0	2	4
Non pathogen			
Alternaria porri (Apa)	0	0	0

*The codes of different antigen and antisera are in the parenthesis.

From Table 17, 18 and Fig. 16 it was evident that the antigen of *C. gloeosporioides* shared one precipitin arc when treated with the antisera of Pusa purple long (PplA). Antigen of Shamala variety and Lalguli variety shared two precipitin arcs when treated with the antisera of Pusa purple long (PplA). Antigens of Pusa purple long, Pusa purple round, Pant brinjal-4, and PK-123 shared four precipitin bands each in all the cases when reacted with antisera of Pusa purple long (PplA).

Table 18. Immunoelectrophoretic test of antigens and antisera of brinjal varieties and *Colletotrichum gloeosporioides*

Antigen of pathogen, host and non-pathogen	Antisera of *C. gloeosporioides* (CgA)				Antisera of pusa purple long (PplA)				Antisera of shamala variety (ShavA)			
	Precipitation arcs				Precipitation arcs				Precipitation arcs			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Pathogen												
C. gloeosporioides (Cga)	+	+	+	–	+	–	–	–	–	–	–	–
Susceptible varieties												
Pusa purple long (Ppla)	+	–	–	–	+	+	+	+	+	+	–	–
Pusa purple round (Ppra)	+	–	–	–	+	+	+	+	+	+	+	–
Pant brinjal-4 (Pb4-a)	+	–	–	–	+	+	+	+	+	+	–	–
PK-123 (P123a)	+	–	–	–	+	+	+	+	+	+	–	–
Resistant varieties												
Shamala variety (Shava)	–	–	–	–	+	+	–	–	+	+	+	+
Lalguli variety (Lva)	–	–	–	–	+	+	–	–	+	+	–	–
Preeti variety (Pva)	–	–	–	–	+	+	–	–	+	+	+	
Kuroi variety (Kva)	–	–	–	–	+	+	–	–	+	+	+	+
Non pathogen												
Alternaria porri (Apa)	–	–	–	–	–	–	–	–	–	–	–	–

Common precipitation band present (+)

Common precipitation band absent (–)

* The codes of different antigens and antisera are in the parenthesis.

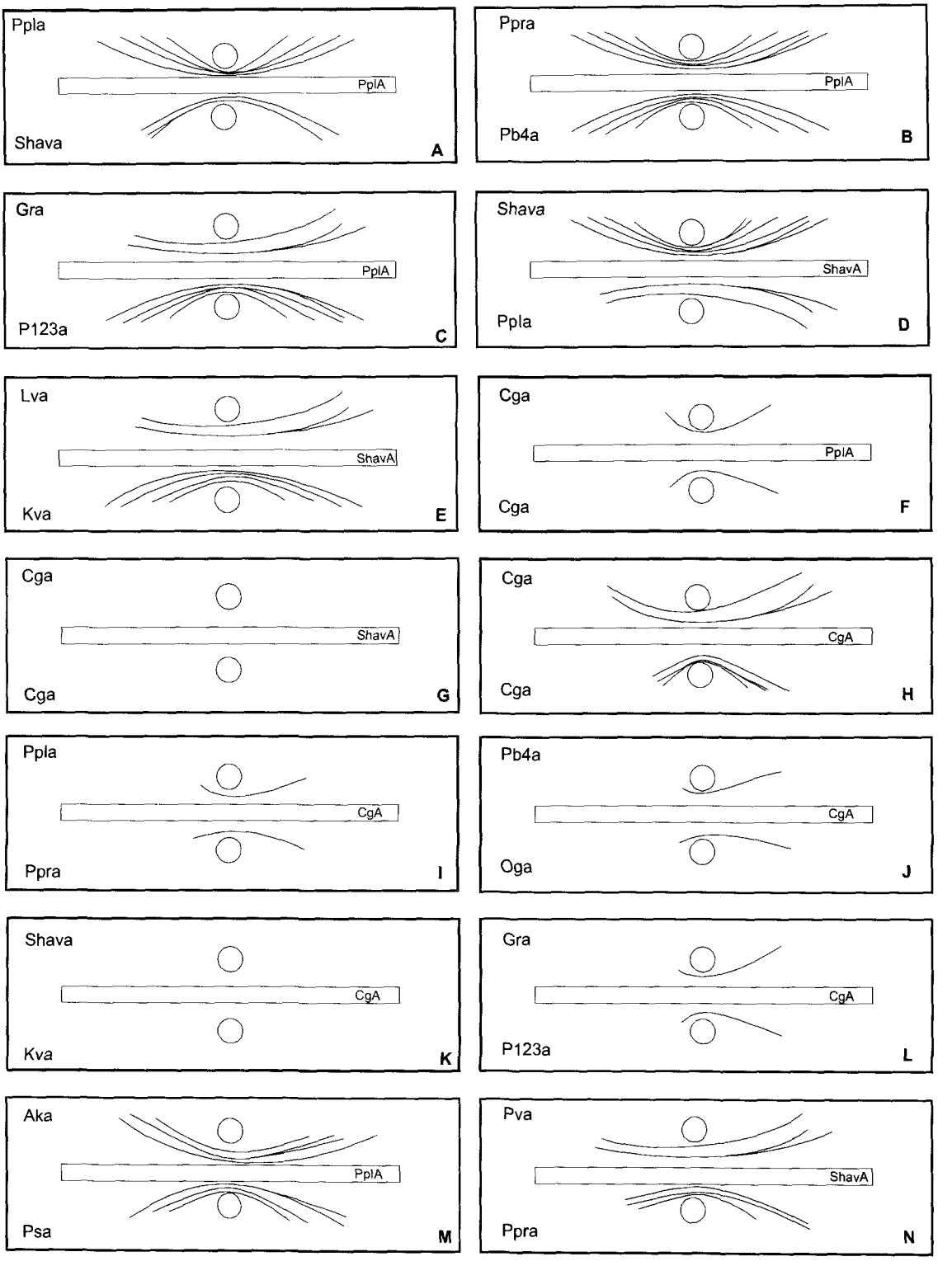

Fig. 16 (A-N) : Immunogram showing immunoelectrophoretic patterns of antigens and antisera of *C. gloeosporioides* and brinjal varieties. Different antisera used in the central rectangular trough are of *C. gloeosporioides* (CgA), Pusa purple long (PplA) and Shamaia variety (ShavA) while different antigens were used in the wells. These are Pusa purple long (Ppla), Pusa purple round (Ppra), Pant brinjal 4 (Pb4a), PK-123 (P123a), Green round (Gra), Shamaia variety (Shava), Orissa green (Oga), Kuroi variety (Kva), Arka keshav (Aka), Pant samrat (Psa), Preeti variety (Pva), Lalguli variety (Lva) and *C. gioeosporioides* (Cga).

Antigens of Pusa purple long, Pusa purple round, Pant brinjal-4, Orissa green and Green round shared one precipitin band each in all the cases when reacted with antisera of *C. gloeosporioides* (CgA) while antigens of Shamala variety, Lalguli variety, Preeti variety and Kuroi variety showed no precipitin band. Antigen of *C. gloeosporioides* shared three precipitin bands with antisera of *C. gloeosporioides* (CgA). Antigen of *C. gloeosporioides* shared one precipitin band with antisera of Pusa purple long (Fig. 16F) although it shared no precipitin band with antisera of Shamala variety (Fig 16G) [Plate XI: A-D].

4.4.3. Indirect enzyme linked immunosorbent assay (Indirect ELISA) between *C. gloeosporioides* and different brinjal varieties

The different formats of enzyme linked immunosorbent assays have become increasingly popular since its discovery and introduction as a diagnostic tool in a practical form. It has been clearly pointed out by several authors (Chakraborty and Saha, 1994; Kratka *et al.*, 2002; Dasgupta *et al.*, 2005) on the basis of their findings that indirect ELISA can serve as a useful technique to detect cross-reactive antigens, which determine the susceptibility or resistance of a host in a host parasite combination. Therefore, it was considered worthwhile to study cross-reactive antigens between *C. gloeosporioides* and brinjal varieties by using indirect ELISA format, since this technique is one of the most sensitive serological technique to detect and quantify low concentration of antigen.

The leaf antigens of all the 28 brinjal varieties included in this study, mycelial antigen of the fungal pathogen *C. gloeosporioides* and a non pathogen fungus *Alternaria porri* were used as antigen while antisera of two brinjal varieties (Pusa purple long and Shamala variety) and the fungal pathogen *C. gloeosporioides* were used to perform indirect ELISA. All the antisera as well as normal sera were diluted in 1/125 dilution and were tested against three different concentrations (5 µg ml^{-1}, 10 µg ml^{-1}, and 20 µg ml^{-1}) of each antigen separately. The detailed procedure of indirect ELISA as well as the preparation of antigens and antisera has already been discussed in the materials and methods (Section 3.13.3). An ELISA reader determined the absorbance of all the combinations at 492 nm and the results are given in Table 19 and Fig. 17.

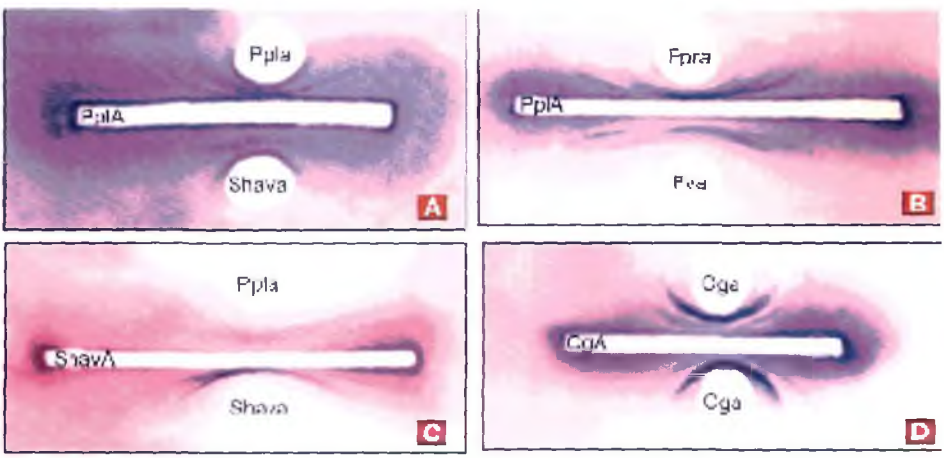

Plate XI

Fig. A-D: Immunoelectrophpresis of antigens and antisera.

Different antigens used in the wells with codes:

Pusa purple long - (Ppla)

Shamala variety - (Shava)

Pusa purple round - (Ppra)

Preeti variety - (Pva)

C. gloeosporioides - (Cga).

Different antisera used in the central rectangular trough with codes:

Pusa purple long - (PplA)

Shamala variety - (ShavA)

C. gloeosporioides - (CgA).

Table19. Indirect ELISA (A_{492}) results of different combination of antigens (antigens of 28 brinjal varieties and *Colletotrichum gloeosporioides*) against three different antisera (antisera of Pusa purple long, Shamala variety and *C. gloeosporioides*).

Antigen of host/pathogen	µg protein /ml	Normal sera (NS) and antisera (AS) of susceptible and resistant brinjal varieties and pathogen					
		Pusa purple long (PplA)		Shamala variety (ShavA)		*C. gloeosporioides* (CgA)	
		NS 125	AS 125	NS 125	AS 125	NS 125	AS 125
Pusa purple long (Ppla)	20	0.030	1.122	0.030	0.759	0.030	0.532
	10	0.027	0.985	0.027	0.736	0.027	0.402
	5	0.023	0.978	0.023	0.723	0.023	0.353
Pusa purple round (Ppra)	20	0.029	1.115	0.029	0.772	0.029	0.520
	10	0.026	0.978	0.026	0.743	0.026	0.399
	5	0.021	0.960	0.021	0.741	0.021	0.335
Pant brinjal-4 (Pb4a)	20	0.031	1.105	0.031	0.791	0.031	0.518
	10	0.028	0.977	0.028	0.768	0.028	0.387
	5	0.027	0.927	0.027	0.743	0.027	0.322
Orissa green (Oga)	20	0.031	1.074	0.032	0.835	0.032	0.450
	10	0.027	0.970	0.027	0.794	0.027	0.377
	5	0.022	0.967	0.022	0.766	0.022	0.297
Green round (Gra)	20	0.029	1.048	0.029	0.841	0.029	0.413
	10	0.027	0.950	0.027	0.822	0.027	0.388
	5	0.022	0.957	0.022	0.796	0.022	0.301
Ppl-74 (P74a)	20	0.032	1.019	0.032	0.849	0.032	0.401
	10	0.029	0.943	0.029	0.824	0.029	0.353
	5	0.026	0.940	0.026	0.806	0.026	0.283
BE-706 (B706a)	20	0.028	1.003	0.028	0.872	0.028	0.387
	10	0.024	0.933	0.024	0.861	0.024	0.351
	5	0.020	0.901	0.020	0.831	0.020	0.320
Supriya variety (Supva)	20	0.029	0.992	0.029	0.883	0.029	0.360
	10	9,925	0.913	0.025	0.872	0.025	0.320
	5	0.022	0.889	0.022	0.843	0.022	0.224
Nisha variety (Nva)	20	0.031	0.977	0.031	0.899	0.031	0.351
	10	0.028	0.898	0.028	0.877	0.028	0.304
	5	0.023	0.886	0.023	0.853	0.023	0.291
Suchitra variety (Sucva)	20	0.028	0.970	0.028	0.915	0.028	0.340
	10	0.025	0.893	0.025	0.893	0.025	0.321
	5	0.020	0.869	0.020	0.877	0.020	0.294

Contd...Table 19

Table 19 (Contd..) Indirect ELISA (A_{492}) results of different combination of antigens (antigens of 28 brinjal varieties and *Colletotrichum gloeosporioides*) against three different antisera (antisera of Pusa purple long, Shamala variety and *C. gloeosporioides*)

Antigen of host/pathogen	µg protein /ml	Normal sera (NS) and antisera (AS) of susceptible and resistant brinjal varieties and pathogen					
		Pusa purple long (PplA)		Shamala variety (ShavA)		*C. gloeosporioides* (CgA)	
		NS 125	AS 125	NS 125	AS 125	NS 125	AS 125
Pant rituraj (Pra)	20	0.030	0.936	0.030	0.929	0.030	0.332
	10	0.027	0.862	0.027	0.904	0.027	0.282
	5	0.024	0.851	0.024	0.883	0.024	0.269
Green long (Gla)	20	0.025	0.916	0.025	0.955	0.025	0.329
	10	0.023	0.850	0.023	0.937	0.023	0.282
	5	0.200	0.846	0.020	0.913	0.020	0.227
PK-123 (P123a)	20	0.031	0.904	0.031	0.967	0.031	0.328
	10	0.026	0.841	0.026	0.942	0.026	0.286
	5	0.024	0.821	0.024	0.928	0.024	0.254
BSS-465 chhaya (B465-a)	20	0.030	0.892	0.030	0.972	0.030	0.325
	10	0.024	0.731	0.024	0.951	0.024	0.312
	5	0.022	0.818	0.022	0.933	0.022	0.228
Arka keshav (Aka)	20	0.030	0.885	0.030	0.987	0.030	0.321
	10	0.028	0.725	0.028	0.966	0.028	0.308
	5	0.027	0.793	0.027	0.942	0.027	0.233
Kanha hybrid (Kha)	20	0.029	0.880	0.029	0.993	0.029	0.300
	10	0.025	0.710	0.025	0.971	0.025	0.273
	5	0.020	0.793	0.020	0.944	0.020	0.241
BSS-330 (B330a)	20	0.030	0.872	0.030	1.002	0.030	0.299
	10	0.024	0.795	0.024	0.983	0.024	0.273
	5	0.021	0.785	0.021	0.963	0.021	0.256
Agrayani variety (Ava)	20	0.024	0.862	0.024	1.043	0.024	0.298
	10	0.022	0.790	0.022	1.017	0.022	0.278
	5	0.020	0.760	0.020	0.994	0.020	0.249
Nirupama hybrid (Nha)	20	0.028	0.859	0.028	1.057	0.028	0.295
	10	0.026	0.796	0.026	0.032	0.026	0.262
	5	0.022	0.764	0.022	1.017	0.022	0.246
BSS-470 janam (B470a)	20	0.029	0.845	0.029	1.068	0.029	0.273
	10	0.025	0.789	0.025	1.035	0.025	0.259
	5	0.024	0.763	0.024	1.022	0.024	0.242

Contd...Table 19

Table 19 (Contd..) Indirect ELISA (A_{492}) results of different combination of antigens (antigens of 28 brinjal varieties and *Colletotrichum gloeosporioides*) against three different antisera (antisera of Pusa purple long, Shamala variety and *C. gloeosporioides*)

Antigen of host/pathogen	µg protein /ml	Normal sera (NS) and antisera (AS) of susceptible and resistant brinjal varieties and pathogen					
		Pusa purple long (PplA)		Shamala variety (ShavA)		*C. gloeosporioides* (CgA)	
		NS 125	AS 125	NS 125	AS 125	NS 125	AS 125
Pant samrat (Psa)	20	0.027	0.850	0.027	1.071	0.027	0.268
	10	0.024	0.754	0.024	1.049	0.024	0.244
	5	0.020	0.753	0.020	1.040	0.020	0.239
Baromasi local (Bla)	20	0.031	0.842	0.031	1.082	0.031	0.258
	10	0.030	0.797	0.030	1.063	0.030	0.234
	5	0.026	0.747	0.026	0.041	0.026	0.229
Muktakeshi local (Mla)	20	0.030	0.811	0.030	1.085	0.030	0.257
	10	0.025	0.786	0.025	1.065	0.025	0.255
	5	0.023	0.740	0.023	1.053	0.023	0.345
Kuroi variety (Kva)	20	0.029	0.806	0.029	1.097	0.029	0.241
	10	0.025	0.781	0.025	1.088	0.025	0.213
	5	0.023	0.728	0.023	1.054	0.023	0.200
Aam begun (Aba)	20	0.030	0.776	0.030	1.101	0.030	0.212
	10	0.026	0.774	0.026	1.089	0.026	0.210
	5	0.023	0.719	0.023	1.059	0.023	0.165
Preeti variety (Pva)	20	0.025	0.758	0.025	1.113	0.025	0.193
	10	0.024	0.737	0.024	1.097	0.024	0.175
	5	0.021	0.717	0.021	1.063	0.021	0.157
Lalguli variety (Lva)	20	0.026	0.731	0.026	1.119	0.026	0.192
	10	0.023	0.698	0.023	1.102	0.023	0.182
	5	0.021	0.689	0.021	1.075	0.021	0.178
Shamala variety (Shava)	20	0.030	0.713	0.030	1.135	0.030	0.185
	10	0.027	0.698	0.027	1.107	0.027	0.178
	5	0.025	0.687	0.025	1.082	0.025	0.177
C. gloeosporioides (Cga)	20	0.029	0.955	0.029	0.221	0.029	1.025
	10	0.026	0.912	0.026	0.194	0.026	0.957
	5	0.024	0.874	0.024	0.182	0.024	0.900
Non-Pathogen *Alternaria porri* (Apa)	20	0.028	0.149	0.028	0.154	0.029	0.163
	10	0.025	0.238	0.025	0.144	0.025	0.155
	5	0.023	0.124	0.023	0.139	0.023	0.136

*Codes of antigens and antisera are in the parenthesis.

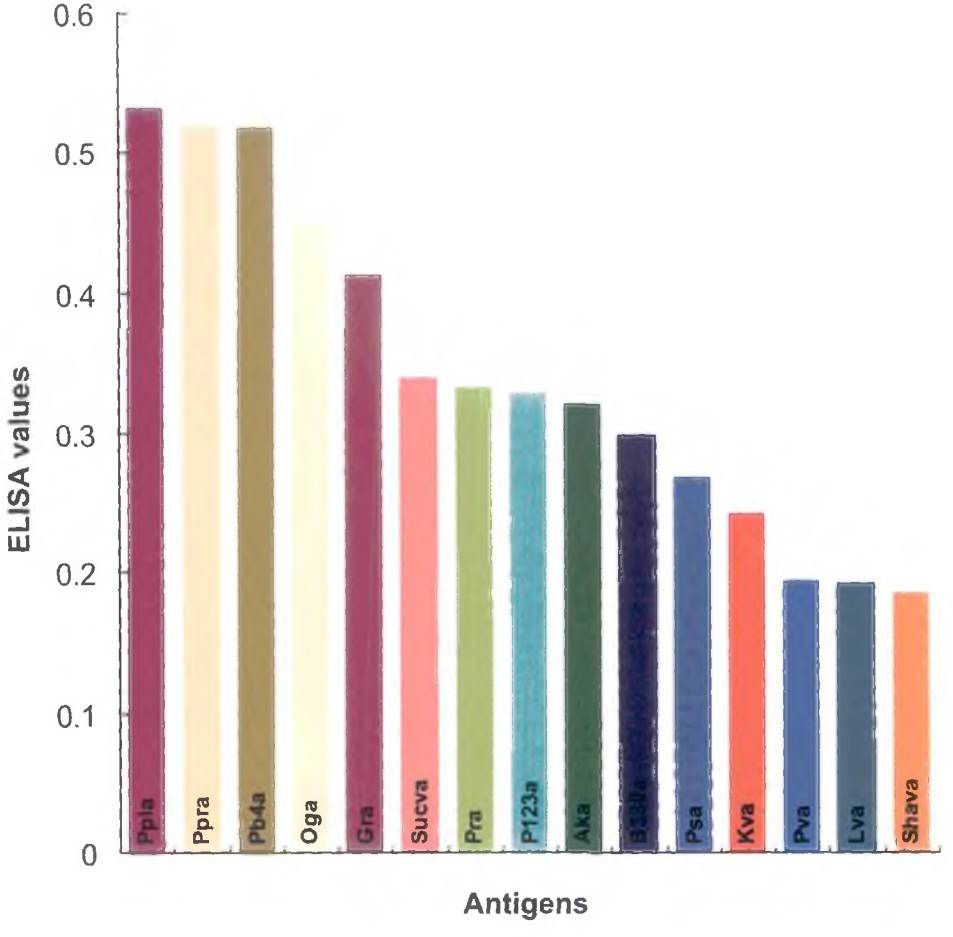

Fig. 17 : Indirect ELISA (A_{492}) results of different combination of antigens [antigens of different brinjal varieties e.g. Pusa purple long (Ppla), Pusa purple round (Ppra), Pant brinjal-4 (Pb4a), Orissa green (Oga), Green round (Gra), Suchitra variety (Sucva), Pant rituraj (Pra), PK-123 (P123a), Arka keshav (Aka), Bss-330 (B330a), Pant samrat (Psa), Kuroi variety (Kva), Preeti variety (Pva), Lalguli variety (Lva) and Shamala variety (Shava)] against three different antisera [antisera of Pusa purple long (PplA), Shamala variety (ShavA) and *C. gloeosporioides* (CgA)].

From Table 19, it was clear that all the three concentrations of the antigen *C. gloeosporioides* showed higher absorbance values when tested with the antisera of the susceptible variety, Pusa purple long (0.955 at antigen concentration 20 µg/ml) than when tested with antisera of the resistant Shamala variety (0.221 at antigen concentration 20 µg/ml). The reciprocal cross of this combination also showed higher absorbance values produced by antigens of Pusa purple long (0.532 at antigen concentration 20 µg/ml) than produced by antigens of Shamala variety (0.185 at antigen concentration 20 µg/ml) when tested with antisera of *C. gloeosporioides*. This clearly indicates that cross-reactivity was higher between pathogen and susceptible variety than between pathogen and resistant variety. Results obtained from all the combinations showed that the absorbance values of normal serum control were lower than the corresponding test values.

4.4.4. Immunogold labelling for cellular location of antigens and cross-reactive antigens

Immunogold labeling and electron microscopy is a powerful tool for cellular location of different proteins or antigens (Lee *et al.*, 2000; Trillas *et al.*, 2000 and Nahalkova *et al.*, 2001). To visualize immunogold labels in light microscope, silver enhancement is essential. Colloidal gold labels are normally visible only at electron microscope level. Silver enhancer enhances the colloidal gold label by precipitation of metallic silver to give a high contrast signal visible under light microscope. Fluorescent antibody labeling with fluorescein isothiocyanate (FITC) is also known to be one of the powerful techniques to determine the cell or tissue location of antigens or proteins. However, autofluorescence present in the plant tissues may mislead the proper understanding of the actual cellular location of CRA or any proteins. Moreover, a fluorescence microscope is more expensive than light microscope. Hence it was considered worthwhile to perform immunogold labeling followed by silver enhancement for tissue location of CRA under light microscope.

Earlier serological experiments like immunodiffusion, immunoelectrophoresis and indirect enzyme linked immunosorbent assay (indirect ELISA) clearly indicated the presence of cross reactive antigens (CRA) between brinjal varieties and *C. gloeosporioides*. To find out tissue and cellular location of CRA shared by the pathogen and brinjal leaves, immunogold labeling studies followed by silver enhancement were performed. Leaf sections (cut through midrib) of susceptible (Pusa purple long) and resistant (Shamala variety) brinjal varieties and mycelia and

spores of *C. gloeosporioides* were used as antigens. The antisera of Pusa purple long (susceptible variety), Shamala variety (resistant variety) and the pathogen *C. gloeosporioides* were used in the experiment. To determine the exact location of CRA, both leaf section and fungal mycelia were treated with antisera and subsequently immunogold labeling and silver enhancement of the host and pathogen was done. The procedure has been discussed in the materials and methods section in details (Section 3.13.4).

Normal untreated section of brinjal leaf when observed under light microscope, showed no precipitation (Plate XII A and Plate XIII A) and the cells were greenish in colour. When immunogold labeling and silver enhancement were performed on leaf sections that were treated with normal sera the natural greenish color disappeared but no precipitation was observed on the cells (Plate XII B). Leaf sections of pusa purple long when treated with antisera of Pusa purple long (i.e. with homologous antisera) and subsequent immunogold labeling followed by silver enhancement, showed maximum precipitation in the epidermal regions, mesophyll tissues and vascular bundle elements of the leaves (Plate XII C and Plate XII D). Similar result was observed when leaf sections of Shamala variety were treated with homologous antisera (Plate XIII C and Plate XIII D). Heavy precipitation was also observed when leaf sections of Pusa purple long was treated with antisera of Shamala variety and vice versa (Plate XII E and Plate XIII E).

In heterologous reaction, when the leaf section of susceptible variety (Pusa purple long) was treated with antisera of *C. gloeosporioides* and labeled with immunogold particles enhanced by silver precipitation, darkening was observed mainly in the epidermal regions. Some precipitation was also found distributed in mesophyll tissues and vascular bundle elements but these were comparatively less dark than observed for homologous reaction (Plate XII F) indicating the presence of CRA. When leaf section of resistant variety (Shamaia) was treated with the antisera of pathogen (*C. gloeosporioides*) faint precipitation was observed after immunogold labeling and silver enhancement (Plate XIII F).

Immunogold labeling and silver enhancement of mycelia and spores of the pathogen *C. gloeosporioides* showed that these were grayish in normal condition (Plate XIV A). When treated with antisera of *C. gloeosporioides* (i.e. homologous treatment) mycelia and spores took dark color after immunogold labeling and silver enhancement (Plate XIV B). When treated with antisera of susceptible variety (Pusa

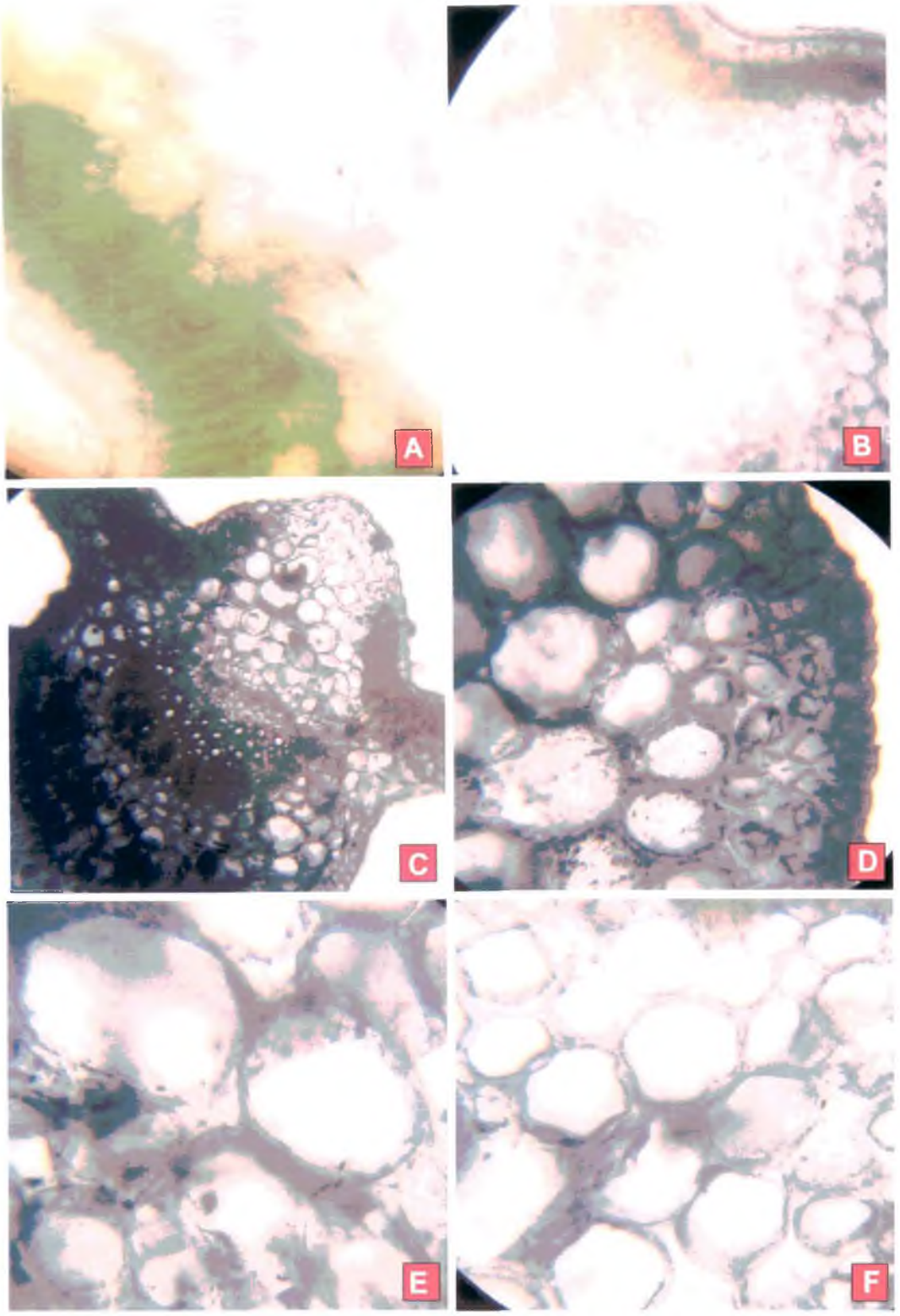

Plate XII : Immunogold labelling and silver enhancement of brinjal leaf tissues for detection of cross-reactive antigens and homologous antigens.

Fig. A : Untreated leaf section of Pusa purple long (Ppl).

Fig. B : Leaf section of Pusa purple long (Ppl) treated with normal sera.

Fig. C : Leaf section of Pusa purple long (Ppl) treated with antisera of Pusa purple long (PplA).

Fig. D : Leaf section of Pusa purple long (Ppl) treated with antisera of Pusa purple long (PplA).

Fig. E : Leaf section of Pusa purple long (Ppl) treated with antisera of Shamala variety (ShavA).

Fig. F : Leaf section of Pusa purple long (Ppl) treated with antisera of *C. gloeosporioides*.

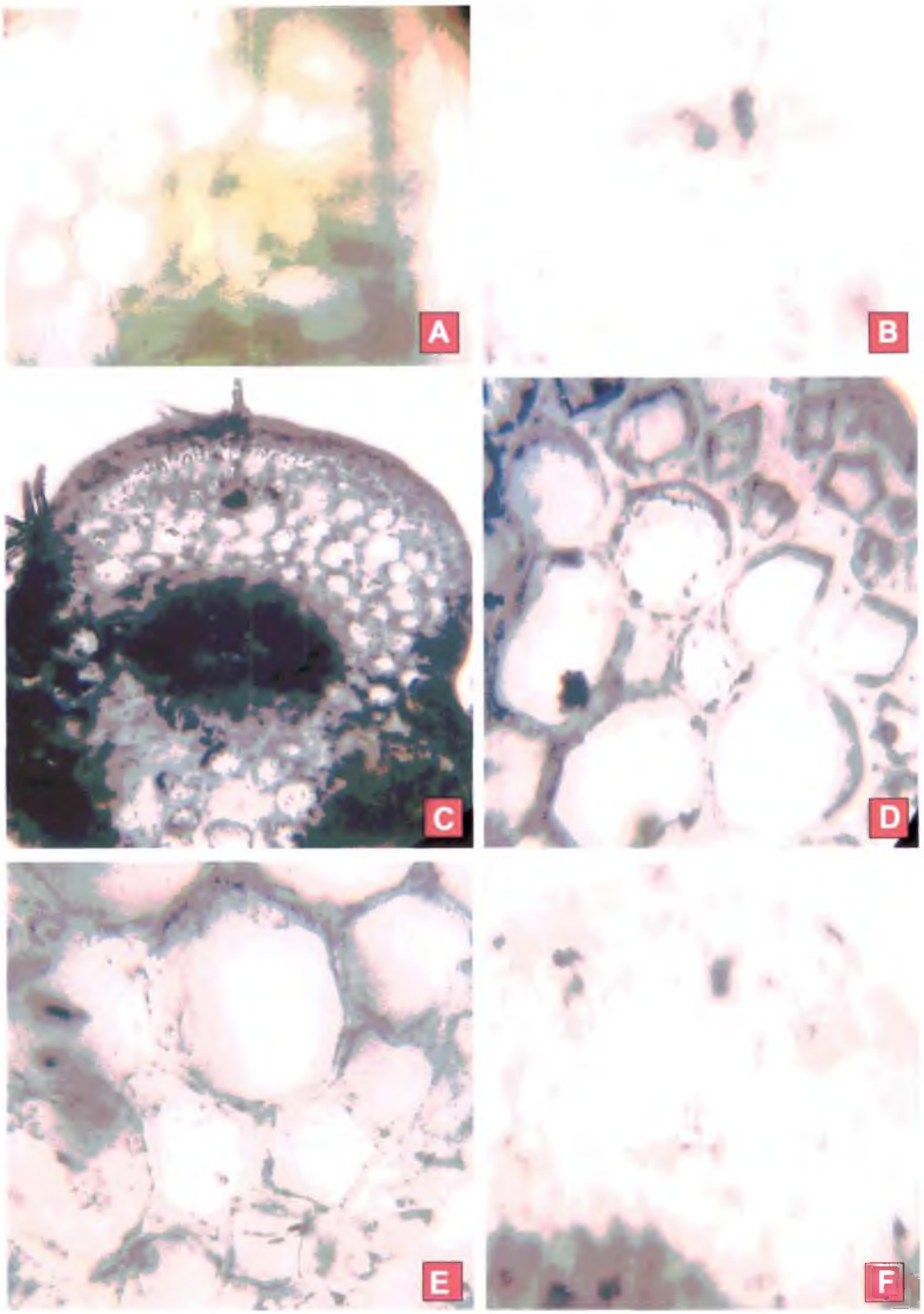

Plate XIII : Immunogold labelling and silver enhancement of brinjal leaf tissues for detection of cross-reactive antigens and homologous antigen.

Fig. A : Untreated leaf section of Shamala variety (Shav).

Fig. B : Leaf section of Shamala variety (Shav) treated with normal sera.

Fig. C : Leaf section of Shamala variety (Shav) treated with antisera of Shamala variety (ShavA).

Fig. D : Leaf section of Shamala variety (Shav) treated with antisera of Shamala variety (ShavA).

Fig. E : Leaf section of Shamala variety (Shav) treated with antisera of Pusa purple long (PplA).

Fig. F : Leaf section of Shamala variety (Shav) treated with antisera of *C. gloeosporioides* (CgA).

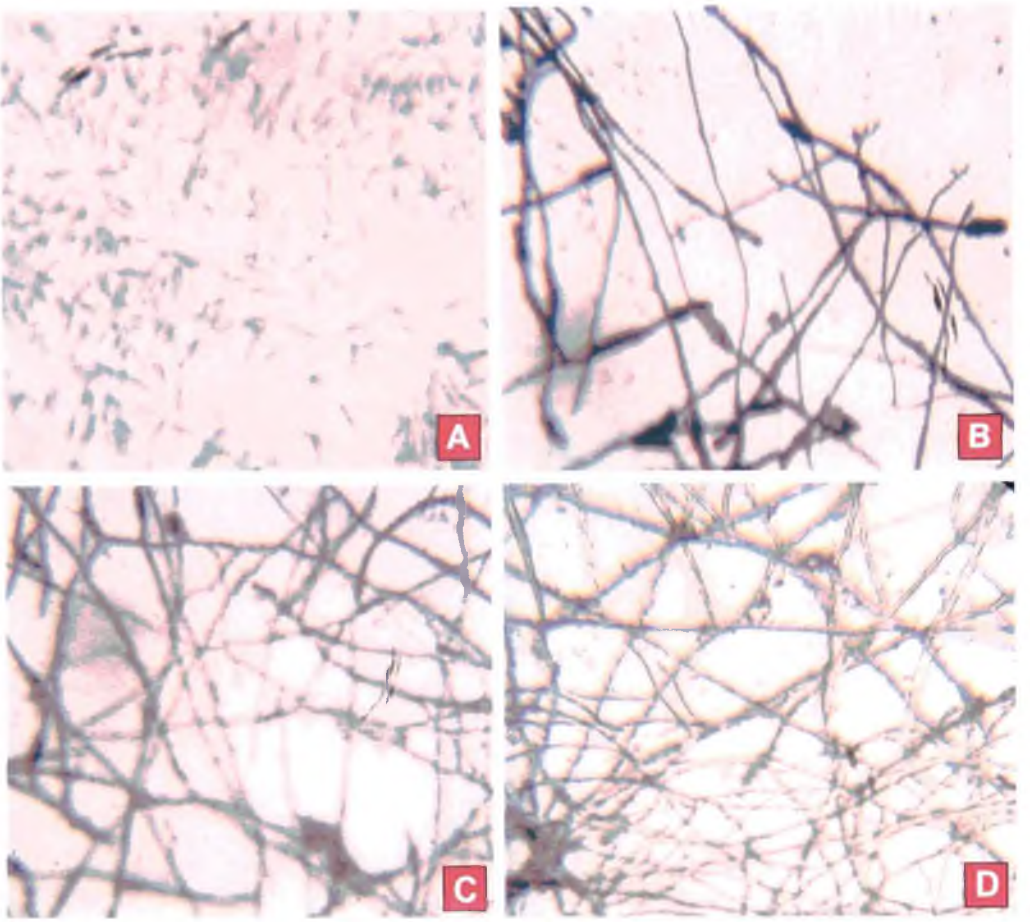

Plate XIV : Immunogold labelling and silver enhancement of spores and mycelia of *Colletotrichum gloeosporioides* for detection of cross-reactive antigens and homologous antigen.

Fig. A : Untreated spores of *C. gloeosporioides*.

Fig. B : Spores and mycelia of *C. gloeosporioides* treated with antisera of *C. gloeosporioides* (CgA).

Fig. C : Mycelia of *C. gloeosporioides* treated with antisera of Pusa purple long (PplA).

Fig. D : Mycelia of *C. gloeosporioides* treated with antisera of Shamala variety (ShavA).

purple long) mycelia showed darkening mainly in the hyphal tips and patches in mycelium and spores, but not as much as homologous reaction (Plate XIV C). Spores and mycelia when treated with antisera of resistant variety (Shamala) showed almost no precipitation and retained normal grayish color (Plate XIV D).

4.5. Alteration of disease reaction following application of various chemicals in selected brinjal varieties against *C. gloeosporioides*

Disease manifestation may be altered by using exogenous chemicals which act as elicitors of defense response. Plants thereby systemically develop resistance to diseases known as systemic acquired resistance (Kessman *et al.*, 1994; Ryals *et al.*, 1994). Development of resistance provides a long term disease control strategy and was therefore included in the present study.

4.5.1. Various chemical treatments for Induction of resistance

For induction of systemic acquired resistance (SAR), several chemicals have been recorded which helps the plants to resist the pathogen attack by altering the disease reaction. Hence to control the anthracnose pathogen *C. glepsporioides* in brinjal plant, the following work was undertaken. Chemicals like ferric chloride, salicylic acid, indole acetic acid, indole butyric acid, sodium molybdate, 2,3-dihydroxy benzoic acid, 2,1,3-benzothiadiazole, 4-amino butyric acid, 2-amino butyric acid and sodium azide were selected for spraying at three different concentrations (10^{-3} M, 10^{-4} M and 10^{-5} M) following the procedure as described in materials and methods (Section 3.14.). Along with these chemicals, commercially available jasmonic acid at 10^{-3} M concentration was also sprayed. Fresh aqueous leaf extracts (2 g fresh weight / 10 ml of distilled water) of *Jasminum jasminoides, Acalypha indica, Melia dubia, Azadirachta indica* and *Catharanthus roseus* were also sprayed. Plants were inoculated with the spore suspension of *C. gloeosporioides* after 24 hour of spraying by whole plant inoculation technique (Section 3.6.2). Results were tabulated in Table 20 and Table 21.

Table 20. Induction of disease resistance in susceptible brinjal variety (Pusa purple long) against *C. gloeosporioides* following treatment of different chemicals.

Chemicals	Concentration	Mean disease index / plant *		
		Incubation period		
		6 days	9 days	12 days
Ferric chloride	10^{-3} M	5.11±0.39	7.65±0.33	9.71±0.42
	10^{-4} M	6.57±0.49	8.36±0.40	10.48±0.43
	10^{-5} M	4.37±0.36	6.15±0.32	8.37±0.40
Salicylic acid	10^{-3} M	7.97±0.36	12.01±0.63	15.74±0.44
	10^{-4} M	4.56±0.39	5.67±0.23	8.68±0.33
	10^{-5} M	6.79±0.46	11.77±0.38	14.69±0.41
Jasmonic acid	10^{-3} M	1.83±0.15	4.00±0.09	4.90±0.21
Indole acetic acid	10^{-3} M	8.07±0.39	13.60±0.22	16.72±0.30
	10^{-4} M	7.32±0.36	13.08±0.32	16.14±0.26
	10^{-5} M	5.86±0.36	9.59±0.29	11.49±0.41
Indole butyric acid	10^{-3} M	7.56±0.23	13.00±0.42	17.24±0.48
	10^{-4} M	6.82±0.29	11.80±0.24	16.57±0.39
	10^{-5} M	4.81±0.21	8.64±0.48	9.88±0.57
Sodium molybdate	10^{-3} M	3.54±0.23	4.93±0.20	8.86±0.40
	10^{-4} M	2.45±0.16	4.21±0.19	7.67±0.34
	10^{-5} M	3.09±0.18	5.60±0.06	8.25±0.25
2,3-dihydroxy benzoic acid	10^{-3} M	5.62±0.32	8.79±0.27	11.46±0.38
	10^{-4} M	6.13±0.29	9.88±0.44	14.42±0.23
	10^{-5} M	7.14±0.36	12.10±0.49	17.18±0.48
2,1,3-benzothiadiazole	10^{-3} M	7.93±0.24	14.06±0.37	17.00±0.30
	10^{-4} M	4.45±0.22	8.63±0.35	9.31±0.22
	10^{-5} M	6.20±0.35	9.91±0.45	12.68±0.33
4-Amino butyric acid	10^{-3} M	5.63±0.26	7.57±0.26	9.01±0.33
	10^{-4} M	3.36±0.26	5.59±0.18	6.61±0.36
	10^{-5} M	6.39±0.32	10.71±0.31	15.76±0.61
2-Amino butyric acid	10^{-3} M	6.76±0.27	11.77±0.47	15.24±0.39
	10^{-4} M	7.01±0.24	12.09±0.44	16.27±0.37
	10^{-5} M	7.09±0.34	12.24±0.34	16.23±0.35
Sodium Azide	10^{-3} M	4.97±0.28	6.11±0.28	7.62±0.31
	10^{-4} M	3.16±0.20	4.68±0.33	5.34±0.27
	10^{-5} M	4.08±0.21	5.66±0.28	6.62±0.39
Control		7.16±0.68	12.50± 0.41	17.00±0.39
CD at 5%		0.21	0.25	0.33

* Mean of 3 replications. Data after ± represent standard error values.

Table 21. Induction of disease resistance in susceptible brinjal variety (Pusa purple long) against *C. gloeosporioides* following treatment with plant extracts.

Plant extracts**	Mean disease index / plant *		
	Incubation period (Days)		
	6	9	12
Jasminum jasminoides	2.11±0.18	4.36±0.21	5.46±0.32
Acalypha indica	7.19±0.31	12.07±0.29	15.63±0.57
Melia dubia	6.76±0.26	11.36±0.29	15.26±0.35
Azadirachta indica	5.64±0.13	7.19±0.14	10.03±0.39
Catharanthus roseus	6.07±0.32	8.12±0.25	11.32±0.39
Control	7.16±0.68	12.50± 0.41	17.00± 0.39
CD at 5%	0.17	0.22	0.31

* Mean of 3 replications. Data after ± represent standard error values.

**Fresh aqueous extracts of leaves (2 g fresh weight / 10 ml distilled water) were used.

From the result it was evident that out of eleven chemicals tested, only five e.g. 2,1,3-benzo-thiadiazole, 4-amino butyric acid, sodium azide, salicylic acid and jasmonic acid were significantly effective in reducing the disease occurrence (mean disease index/plant) with respect to control. Ferric chloride, sodium molybdate and 2,3-dihydroxy benzoic acid showed marginal reduction in disease induction. Indole acetic acid, indole butyric acid and 2-amino butyric acid showed more or less same disease index as control. Out of five leaf extracts tested, *J. jasminoides* showed excellent control of the disease followed by *A. indica*. *C. roseus* also reduced mean disease index with respect to control.

After preliminary screening, the chemicals and extracts that were found to be effective in inducing resistance in brinjal against anthracnose were selected for further experiments. Therefore, Jasmonic acid (10^{-3} M), 2,1,3-benzothiadiazole (10^{-4} M), 4-amino butyric acid (10^{-4} M), sodium azide (10^{-4} M), salicylic acid (10^{-4} M) and leaf extracts of *J. jasminoides* and *A. indica* were prepared and sprayed on brinjal plants (Pusa purple long and Shamala variety). The plants were inoculated with the spore suspension of *C. gloeosporioides* after 24 hours of spraying. In control set, plants were sprayed with the spore suspension without any pretreatment. The inoculation was done following the whole plant inoculation technique as described in the materials and methods (Section 3.6.2.). Following the

method of Sinha and Das (1972), mean disease index / plant was calculated after 6, 9 and 12 days and noted in Table 22 and graphically represented in Fig. 18.

Table 22. Disease incidence in susceptible and resistant brinjal varieties (Pusa purple long and Shamala variety) against *C. gloeosporioides* following treatment of selected chemicals and plant extracts.

Chemicals/plant extracts*	Brinjal varieties	Mean disease index / plant **		
		Incubation period		
		6 days	9 days	12 days
2,1,3-benzothiadiazole (10^{-4} M)	Ppl	4.47±0.24	8.16±0.28	9.37±0.40
	Shav	0.21±0.03	0.81±0.14	2.03±0.15
Salicylic Acid (10^{-4} M)	Ppl	4.59±0.20	6.01±0.27	9.03±0.71
	Shav	0.20±0.02	0.66±0.06	1.50±0.16
Jasmonic Acid (10^{-3} M)	Ppl	1.89±0.14	3.91±0.40	4.99±0.42
	Shav	0	0.38±0.04	1.05±0.09
4-Amino butyric acid (10^{-4} M)	Ppl	3.07±0.25	5.62±0.37	6.72±0.43
	Shav	0.14±0.02	0.60±0.10	1.60±0.09
Sodium Azide (10^{-4} M)	Ppl	3.11±0.18	4.73±0.41	5.34±0.27
	Shav	0	0.45±0.03	1.38±0.09
Jasminum jasminoides	Ppl	2.09±0.11	4.23±0.39	5.31±0.22
	Shav	0	0.37±0.03	1.14±0.12
Azadirachta indica	Ppl	5.62±0.21	7.20±0.20	10.26±0.45
	Shav	0.15±0.02	0.48±0.05	1.41±0.12
Control	Ppl	7.16±0.39	12.50± 0.41	16.96± 0.37
	Shav	0.24±0.01	0.97±0.05	2.45±0.15
CD at 5%		0.20	0.27	0.31

*Fresh aqueous extracts of leaves (2 g fresh weight / 10 ml distilled water) were used.

** Mean of 3 replications. Data after ± represent standard error values.

Results (Table 22 and Fig. 18) revealed that among the tested elicitors, maximum reduction in disease occurrence was produced when plants were pretreated with jasmonic acid before inoculation. Mean disease index/plant (disease occurrence) was 4.99 in Pusa purple long (susceptible variety) when they were

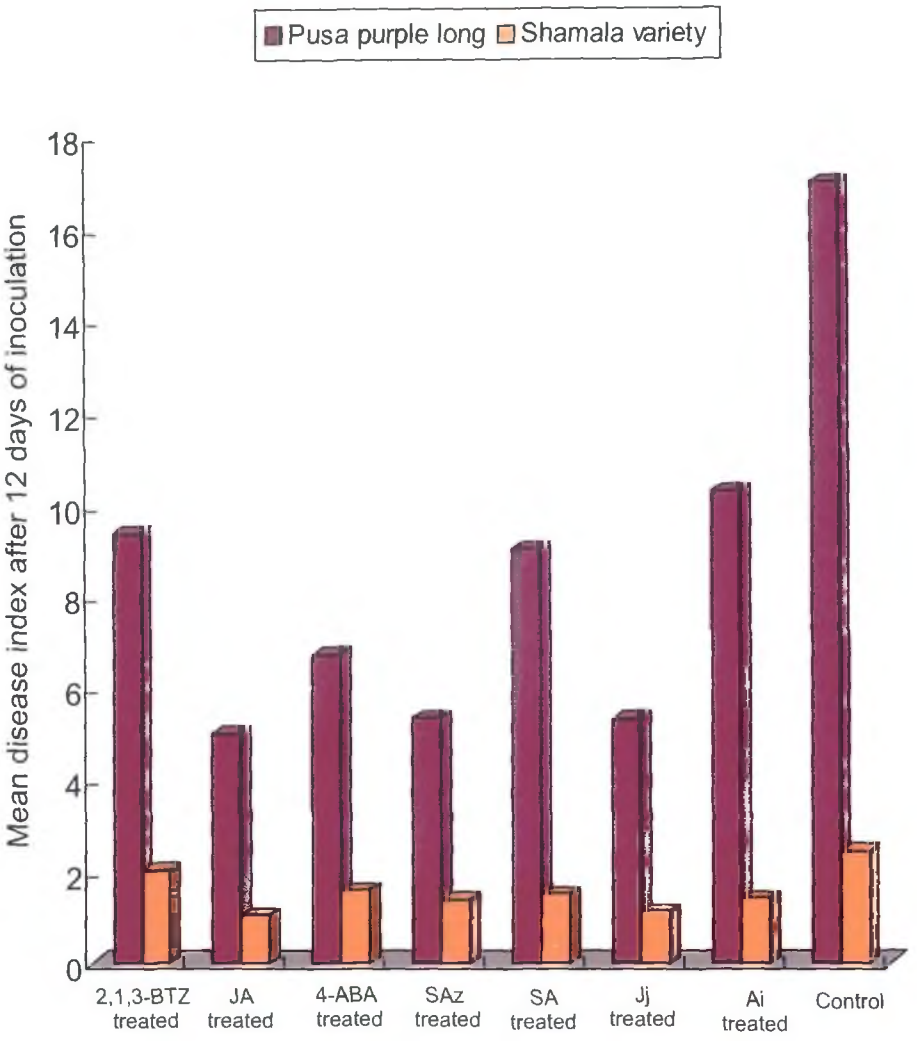

Fig. 18 : Disease occurrence in susceptible (Pusa purple long) and resistant (Shamala variety) brinjal varieties against *C. gloeosporioides* following treatement with some selected chemicals and plant extracts.

Abbreviations: 2,1,3-BTZ = 2,1,3-benzothiadiazole
JA = Jasmonic Acid
4-ABA = 4-Amino butyric acid
SAz = Sodium azide
SA = Salicylic Acid
Jj = *Jasminum jasminoides*
Ai = *Azadirachta indica*

pretreated with jasmonic acid after 12 d of inoculation while in the control set it was recorded as 16.96 after same period of inoculation. Under similar condition, in case of Shamala variety (resistant variety), the value was 1.05 which was also lower than the value recorded for control (2.45). Application of 4-amino butyric acid also caused significant reduction where the mean disease index/plant was 6.72 in Pusa purple long and 1.6 in Shamala variety after 12 d of inoculation. Among the extracts, *J. jasminoides* was more effective (mean disease index/plant was 5.31 after 12 d of inoculation on Pusa purple long) than *A. indica* which produced minimum reduction of disease occurrence (10.26) among all elicitors tested.

4.5.2. Changes in orthodihydroxy phenol in healthy leaves and leaves inoculated with *C. gloeosporioides*

Brinjal plants of susceptible (Pusa purple long) and resistant (Shamala) varieties were sprayed with the effective concentration of the effective chemicals accordingly to notice the changes in phenol content. Jasmonic acid (10^{-3} M), 2,1,3-benzothiadiazole (10^{-4} M), 4-amino butyric acid (10^{-4} M), sodium azide (10^{-4} M), salicylic acid (10^{-4} M) and leaf extracts of *Jasminum jasminoides* and *Azadirachta indica* were sprayed (10 plants per treatment) and after 24 hours, the pathogen was inoculated. In a separate set, plants were sprayed with chemicals but were not inoculated with the pathogen. These two sets were categorized as inoculated-treated and uninoculated-treated type respectively. Plants were inoculated with the pathogen without any spraying of chemicals or extracts in control sets. Extraction of orthodihydroxy phenol from the leaves was done after 3, 6, 9 and 12 days of inoculation of the pathogen and was estimated in mg g^{-1} fresh weight tissue following the procedure described in materials and methods (Section 3.15.1). Results of the experiments were noted in Table 23 and also graphically represented in Fig. 19.

From the results (Table 23 and Fig. 19) it was evident that the orthodihydroxy phenol contents were maximum in both inoculated and uninoculated-treated leaves of resistant varieties (Shamala variety) and minimum in both inoculated and uninoculated-treated leaves of susceptible varieties (Pusa purple long) after 3, 6, 9 and 12 days of inoculation in all cases. In both cases, control set showed lower amount of orthodihydroxy phenol.

Table 23. Orthodihydroxy phenol content in healthy and *C. gloeosporioides* infected leaves of resistant and susceptible brinjal varieties (Pusa purple long and Shamala variety respectively) following treatment with selected chemicals and plant extracts.

Chemical treatment	Fungal inoculation	Brinjal varieties	Orthodihydroxy Phenol (mg/g)				
			0d	3d	6d	9d	12d
Jasmonic acid	inoculated	Ppl	1.07	1.25	1.63	1.75	1.72
		Shav	1.75	2.00	2.72	2.95	3.10
	uninoculated	Ppl	1.07	1.29	1.70	1.85	1.80
		Shav	1.75	2.05	2.75	2.85	2.70
4-Amino butyric acid	inoculated	Ppl	1.04	1.45	1.63	1.65	1.60
		Shav	1.77	2.00	2.25	2.54	2.75
	uninoculated	Ppl	1.04	1.55	1.72	1.73	1.70
		Shav	1.80	2.05	2.35	2.65	2.50
Sodium Azide	inoculated	Ppl	1.12	1.50	1.80	1.85	1.65
		Shav	1.78	1.70	2.20	2.10	1.95
	uninoculated	Ppl	1.12	1.65	1.94	1.90	1.90
		Shav	1.78	1.95	2.75	2.35	2.20
2,1,3-benzothiadiazole	inoculated	Ppl	1.03	1.20	1.32	1.32	1.28
		Shav	1.70	2.25	2.43	1.85	1.80
	uninoculated	Ppl	1.03	1.25	1.33	1.36	1.35
		Shav	1.70	2.35	2.50	1.90	1.87
Salicylic Acid	inoculated	Ppl	1.10	1.40	1.65	2.11	2.10
		Shav	1.80	1.95	2.00	2.83	2.55
	uninoculated	Ppl	1.10	1.43	1.75	2.20	2.24
		Shav	1.80	2.10	2.24	2.85	2.70
Jasminum jasminoides	inoculated	Ppl	1.07	1.25	1.60	1.75	1.75
		Shav	1.75	2.00	2.70	2.95	3.00
	uninoculated	Ppl	1.07	1.19	1.60	1.65	1.60
		Shav	1.72	1.95	2.65	2.70	2.55
Azadirachta indica	inoculated	Ppl	1.05	1.10	1.50	1.55	1.50
		Shav	1.77	2.05	2.27	2.40	2.35
	uninoculated	Ppl	1.05	1.15	1.45	1.57	1.55
		Shav	1.77	2.10	2.44	2.65	2.95
Control (untreated and inoculated)		Ppl	1.03	1.17	1.29	1.25	1.15
		Shav	1.73	1.88	1.95	2.07	1.96

Ppl = Pusa purple long (Susceptible variety)
Shav = Shamala variety (Resistant variety)

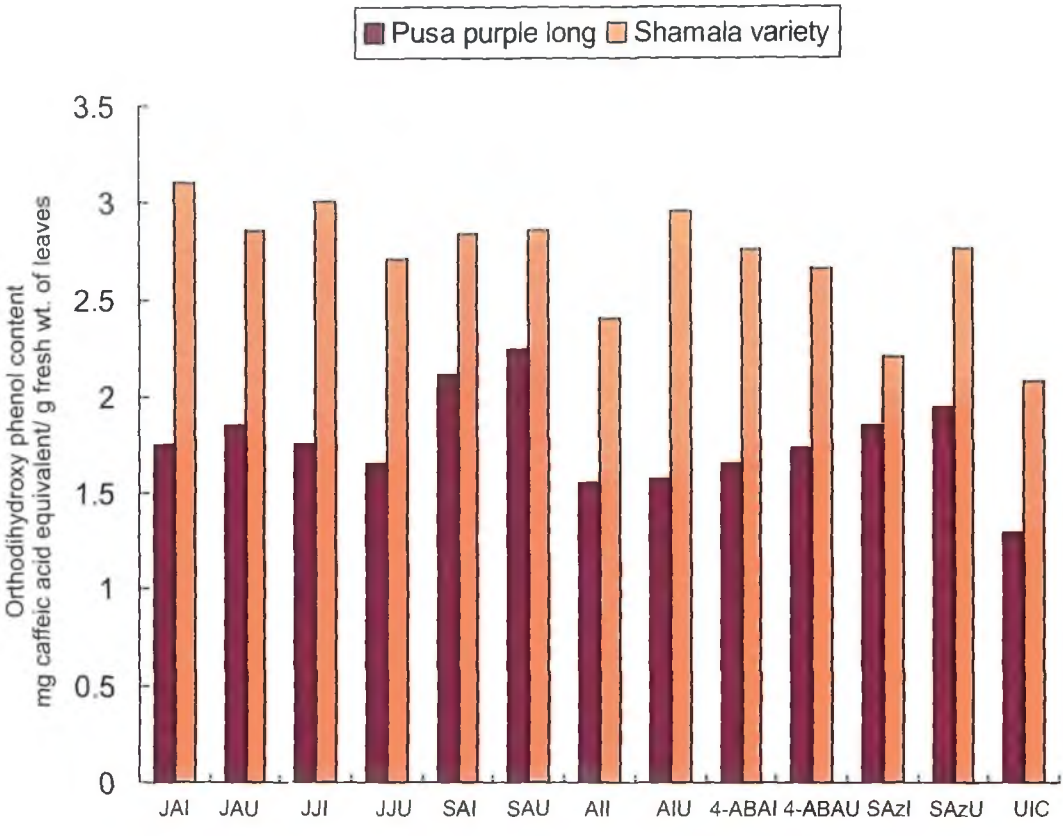

Fig. 19 : Orthodihydroxy phenol content in healthy and *C. gloeosporioides* infected leaves of resistant and susceptible brinjal varieties (Pusa purple long and Shamala variety respectively) following treatment with selected chemicals and plant extracts.

Abbreviations: JAI = Jasmonic acid inoculated
JAU = Jasmonic acid uninoculated
JJI = *Jasminum jasminoides* inoculated
JJU = *Jasminum jasminoides* uninoculated
SAI = Salicylic Acid inoculated
SAU = Salicylic Acid uninoculated
AII = *Azadirachta indica* inoculated
AIU = *Azadirachta indica* uninoculated
4-ABAI = 4, Amino butyric acid inoculated
4-ABAU = 4, Amino butyric acid uninoculated
SAzI = Sodium Azide inoculated
SAzU = Sodium Azide uninoculated
UIC = Control (untreated and inoculated)

4.5.3. Changes in total phenol in healthy leaves and leaves inoculated with *C. gloeosporioides*

Brinjal plants of susceptible (Pusa purple long) and resistant (Shamala variety) varieties were taken and sprayed with the same chemicals as mentioned for estimating changes in orthodihydroxy phenol (Section 4.5.2). The detailed extraction and estimation procedure of total phenol has been discussed under materials and methods (Section 3.15.2). Results obtained from the experiments were noted in the Table 24 and also graphically represented in Fig. 20.

Resistant variety (Shamala) showed maximum values of total phenol content in inoculated-treated leaves while susceptible variety (Pusa purple long) showed minimum values in inoculated-treated leaves following all types of treatement after 3, 6, 9 and 12 days of inoculation in all cases. Total phenol content was also higher in resistant variety than susceptible variety in case of uninoculated-treated leaves in all cases. Total phenol content was higher in both resistant and susceptible varieties in inoculated-treated and uninoculated-treated leaves in comparison to control.

4.6. *In vitro* bioassay of some commonly used fungicides against *C. gloeosporioides*

Application of fungicides to control plant pathogens has been widely accepted by agriculturists for its high success rate, prompt action and easy mode of application. Among a wide range of fungicides, some are found to be more effective than others. Hence, the present work was undertaken to study the *in vitro* effect of six commonly used fungicides in inhibiting the pathogen *C. gloeosporioides.* Five different concentration (50 µg ml $^{-1}$, 125 µg ml $^{-1}$, 250 µg ml $^{-1}$, 500 µg ml $^{-1}$ and 1000 µg ml $^{-1}$) of six selected fungicides e.g. Bavistin, Captan, Indofil, Roko, Baynate and calyxin were taken and bioassay was done following poisoned food technique of Suleman *et al.* (2002) as described under materials and methods (Section 3.16). Initially five different concentrations i.e. 500 µg ml $^{-1}$, 1250 µg ml $^{-1}$, 2500 µg ml $^{-1}$, 5000 µg ml $^{-1}$ and 10000 µg ml $^{-1}$ of all the fungicides were made. Then 9 ml of sterilized medium was prepared and mixed with 1 ml of prepared fungicide solution (made in sterile distilled water) so that the final concentration became 50 µg ml $^{-1}$, 125 µg ml $^{-1}$, 250 µg ml $^{-1}$, 500 µg ml $^{-1}$ and 1000 µg ml $^{-1}$. Each of these concentrations of fungicides were added to molten PDA in petriplates in triplicates.

Table 24. Total phenol content in healthy and *C. gloeosporioides* infected leaves of resistant and susceptible brinjal varieties (Pusa purple long and Shamala variety respectively) following treatment with selected chemicals and plant extracts.

Chemical treatment	Fungal inoculation	Brinjal varieties	Total Phenol (mg/g)				
			0d	3d	6d	9d	12d
Jasmonic acid	inoculated	Ppl	3.55	4.55	6.70	7.60	7.20
		Shav	5.90	7.50	13.10	13.80	13.75
	uninoculated	Ppl	3.55	4.70	6.85	7.40	6.90
		Shav	5.90	6.30	8.60	12.50	8.75
4-Amino butyric acid	inoculated	Ppl	3.55	4.45	4.80	6.22	5.10
		Shav	5.90	6.20	6.50	10.50	6.00
	uninoculated	Ppl	3.55	4.55	4.75	5.90	5.45
		Shav	5.90	6.50	9.50	12.50	6.50
Sodium Azide	inoculated	Ppl	3.55	4.75	5.8	5.20	4.90
		Shav	5.90	6.15	8.00	6.50	6.00
	uninoculated	Ppl	3.55	4.90	6.34	5.55	5.45
		Shav	5.90	7.10	10.50	10.00	9.50
2,1,3-benzo-thiadiazole	inoculated	Ppl	3.55	3.75	4.35	4.05	3.90
		Shav	5.90	7.50	9.75	7.00	5.95
	uninoculated	Ppl	3.55	3.80	4.25	4.10	4.00
		Shav	5.90	6.20	10.50	6.50	6.00
Salicylic Acid	inoculated	Ppl	3.55	4.75	5.50	5.35	5.10
		Shav	5.90	6.00	10.50	9.50	6.00
	uninoculated	Ppl	3.55	4.95	6.70	6.95	5.40
		Shav	5.90	6.50	11.00	10.30	7.50
Jasminum jasminoides	inoculated	Ppl	3.55	4.35	6.50	7.45	7.00
		Shav	5.90	7.00	11.50	12.95	12.80
	uninoculated	Ppl	3.55	4.55	6.75	7.43	6.80
		Shav	5.90	6.30	7.50	11.50	7.75
Azadirachta indica	inoculated	Ppl	3.55	3.95	5.80	6.45	6.40
		Shav	5.90	7.50	10.70	8.10	6.55
	uninoculated	Ppl	3.55	4.10	5.95	6.80	6.35
		Shav	5.90	6.75	11.50	8.75	7.10
Control (untreated and inoculated)		Ppl	3.55	3.80	4.11	3.95	3.83
		Shav	5.90	6.42	7.17	6.75	6.12

Ppl = Pusa purple long (Susceptible variety)

Shav = Shamala variety (Resistant variety)

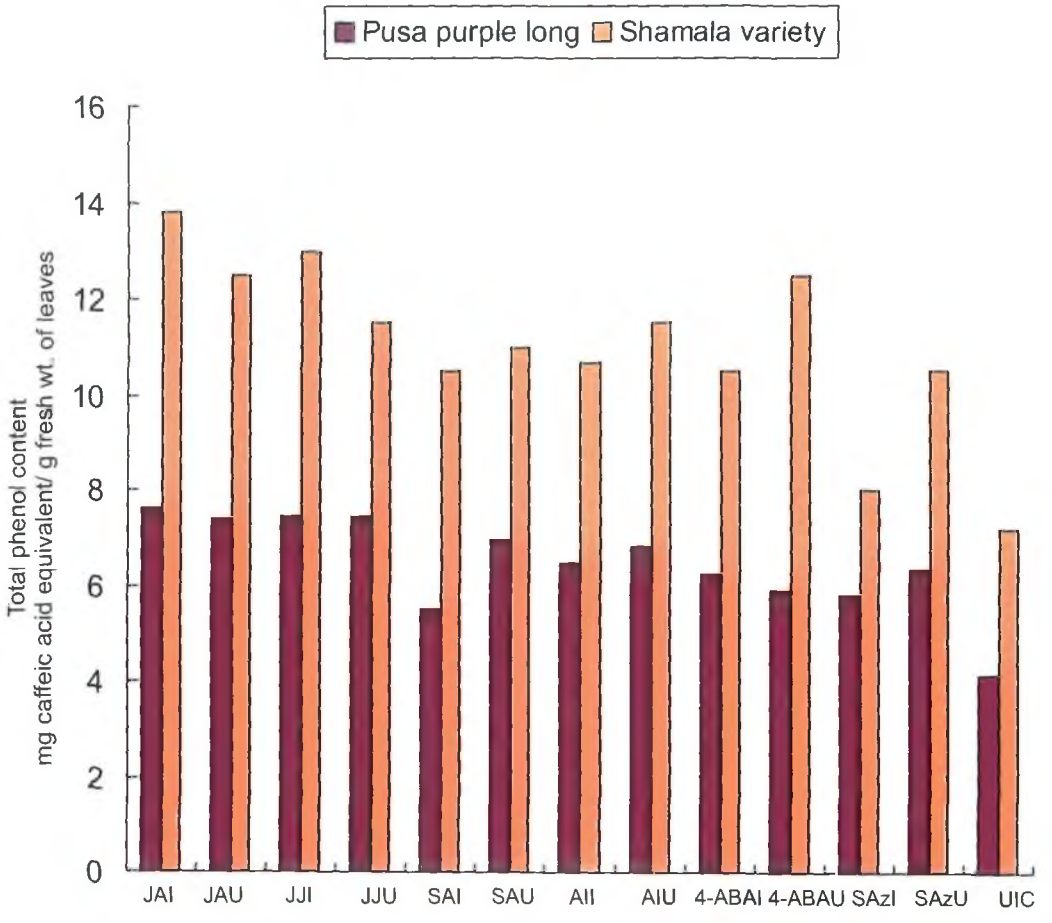

Fig. 20 : Total phenol content in healthy and *C. gloeosporioides* infected leaves of resistant and susceptible brinjal varieties (Pusa purple long and Shamala variety respectively) following treatment with selected chemicals and plant extracts.

Abbreviations:
JAI = Jasmonic acid inoculated
JAU = *Jasmonic acid uninoculated*
JJI = *Jasminum jasminoides* inoculated
JJU = *Jasminum jasminoides* uninoculated
SAI = Salicylic Acid inoculated
SAU = Salicylic Acid uninoculated
AII = *Azadirachta indica* inoculated
AIU = *Azadirachta indica* uninoculated
4-ABAI = 4-Amino butyric acid inoculated
4-ABAU = 4-Amino butyric acid uninoculated
SAzI = Sodium Azide inoculated
SAzU = Sodium Azide uninoculated
UIC = Control (untreated and inoculated)

The plants were inoculated with *C. gloeosporioides* and incubated for the period until which the radial growth in the control plate (PDA without fungicide) touched the edge of the petriplates (90 mm). The percent inhibition over control achieved by each fungicide was recorded (Table 25).

Bioassay studies of the six different fungicides against the pathogen *C. gloeosporioides* (Table 25) showed that at 1000 µg ml $^{-1}$ concentration, almost all the fungicides completely inhibited the growth of the fungus. No fungicide completely inhibited the growth of the fungus at 50 µg ml $^{-1}$ concentration. However, at this concentration, calyxin showed maximum inhibition of growth (61.29%). Only bavistin showed 100% inhibition over control at 250 µg ml $^{-1}$ concentration. However roko, calyxin and baynate containing plate showed no growth at 500 µg ml $^{-1}$ concentration. Baynate showed 49.63% inhibition at 50 µg ml $^{-1}$ concentration. From the result showed on Table 25 it was clear that higher concentration of all the six tested fungicides were effective against the fungus while only calyxin and baynate are effective in lower concentration (50 µg ml $^{-1}$).

4.6.1. Determination of MIC

MIC values of the fungicides tested against the pathogen were determined following the same technique. Ten different concentrations of all the fungicides were prepared by serial dilution and supplemented to the media to get the desired concentration (100 µg ml $^{-1}$, 200 µg ml $^{-1}$, 300 µg ml $^{-1}$, 400 µg ml $^{-1}$, 500 µg ml $^{-1}$, 600 µg ml $^{-1}$, 700 µg ml $^{-1}$, 800 µg ml $^{-1}$, 900 µg ml $^{-1}$ and 1000 µg ml $^{-1}$). Then poisoned food technique (Suleman *et al.*, 2002) was followed according to the procedure described under materials and methods (Section 3.16).

MIC values of the six fungicides (Bavistin, Captan, Indofil, Roko, Baynate and calyxin) against C. *gloeosporioides* were summarized in Table 26. Bavistin was the most effective fungicide among those tested because it showed the lowest MIC value (200 µg ml $^{-1}$). Captan and indofil were least effective with an MIC as high as 900 µg ml $^{-1}$.

Table 25. Effect of different fungicides on growth of *Colletotrichum gloeosporioides*.

Fungicides	Concentration µg/ml	Colony diameter after 7 days of incubation*	Percent inhibition over control**
Bavistin	50	82.00+ 1.53	8.89+1.70
	125	40.17±1.17	55.37±1.30
	250	0	100
	500	0	100
	1000	0	100
Captan	50	86.17+1.17	4.26+1.30
	125	76.17±1.09	15.37±1.22
	250	59.00±2.08	34.44±2.31
	500	36.17±1.17	59.81±1.30
	1000	0	100
Indofil	50	80.00+1.53	11.11+1.70
	125	71.17±1.59	20.92±1.77
	250	53.00±1.15	41.11±1.28
	500	35.33±1.20	60.74±1.33
	1000	0	100
Roko	50	85.50+1.80	5.00+2.00
	125	70.00±2.08	22.22±2.31
	250	34.83±1.59	61.30±1.77
	500	0	100
	1000	0	100
Baynate	50	45.33+1.67	49.63+1.85
	125	31.17±0.60	65.37±0.67
	250	20.17±1.09	77.60±1.21
	500	0	100
	1000	0	100
Calyxin	50	34.83+1.36	61.29+1.52
	125	30.33±2.19	66.29±2.43
	250	16.17±0.73	82.04±0.80
	500	0	100
	1000	0	100
CD at 5%		0.92	1.02

* Mean of three replications. Data after ± represent standard error values.

** Control diameter = 90 mm.

Table 26. MIC values used for controlling *in vivo* radial growth of *Colletotrichum gloeosporioides* following poisoned food technique.

Fungicides	MIC of *C. gloeosporioides* ($\mu g/ml^{-1}$)
Bavistin	200
Captan	900
Indofil	900
Roko	400
Baynate	500
Calyxin	400

4.7. Evaluation of some known biocontrol agents for controlling *C. gloeosporioides*

In recent years, considerable research has been focussed on microbial inoculants that may be used for controlling plant diseases. This is an environment friendly alternative to hazardous fungicides and several workers (Bucki *et al.*,1998; Meena *et al.*, 2000; Ramamoorthy and Samiyappan, 2001; Jadeja, 2003 and Perello *et al.*, 2006) has identified different microorganisms that may be utilized as antagonist to fungal pathogen. Two genera of very commonly used antagonist, one fungus viz. *Trichoderma* and one bacteria viz. *Pseudomonas* has been used in the present study for evaluating their efficacy against the brinjal anthracnose pathogen *C. gloeosporioides.*

4.7.1. *In vitro* screening of some strains of *Trichoderma* and *Pseudomonas*

Initial sceening of 5 *Trichoderma* strains [*Trichoderma viride, T. koningii, T. harzianum, T. virens* (Isolate-I) and *T. virens* (Isolate-II)] and three different isolates of fluorescent pseudomonads for antagonistic potential was done following dual culture test as described in materials and methods (Section 3.17.1). Radial growth and percent inhibition over control were calculated after 5 days of inoculation. The results (Table 27 and Fig. 21) clearly indicated that *Pseudomonas* sp. (Isolate I) was the best biocontrol agent and showed maximum (92.62%) inhibition. Among the fungal antagonists, *T. virens* (Isolate-II) showed maximum (59.76%) growth

inhibition followed by *T. harzianum* (56.90%) and *T. viride* (53.81%) [Plate XV, XVI & XVII].

To control foliar diseases, culture filtrates of fungal antagonists have been utilized for spraying on the infected plants by many workers (Perelló *et al.*,2003 and Brewer and Larkin, 2005). Hence further studies were conducted with crude and cell free culture filtrates of all the tested *Trichoderma* spp. For this, the pathogen was allowed to grow in PDA mixed with culture filtrates (9:1) as described under materials and methods (Section 3.17.2). Radial growth of the test fungs was measured after 5 days of inoculation. Percent inhibitions with respect to control were calculated in each case and the results are tabulated in Table 28.

Table 27. Effect of antagonist on the growth of *Colletotrichum gloeosporioides* tested *in vivo* by dual culture technique.

Antagonists	Colony diameter (mm) of *C. gloeosporioides* after 5 days of incubation*	Percent inhibition over control**
Trichoderma viride	32.33±1.20	53.81±1.71
T. koningii	48.00±1.53	31.43±2.18
T. harzianum	30.17±0.93	56.90±1.32
T. virens (Isolate I)	36.17±1.74	48.33±2.49
T. virens (Isolate II)	28.17±0.93	59.76±1.33
Pseudomonas sp (Isolate I)	5.17±0.17	92.62±0.24
Pseudomonas sp (Isolate II)	35.50±1.26	49.29±1.80
Pseudomonas sp (Isolate III)	38.67±1.20	44.76±1.72
CD at 5%	2.26	3.22

* Mean of three replications. Data after ± represent standard error values.
**Control diameter = 70 mm after 5 d of incubation.

Results of experiments where cell-free culture filtrates were tested (Table 28 and Fig. 22) showed that *T. virens* (Isolate II) produced least colony diameter (28.17 mm) and maximum inhibition (55.96%) followed by *T. harzianum* (53.57%). Least inhibitory effect was shown by culture filtrates of *T. koningii* (14.53%).

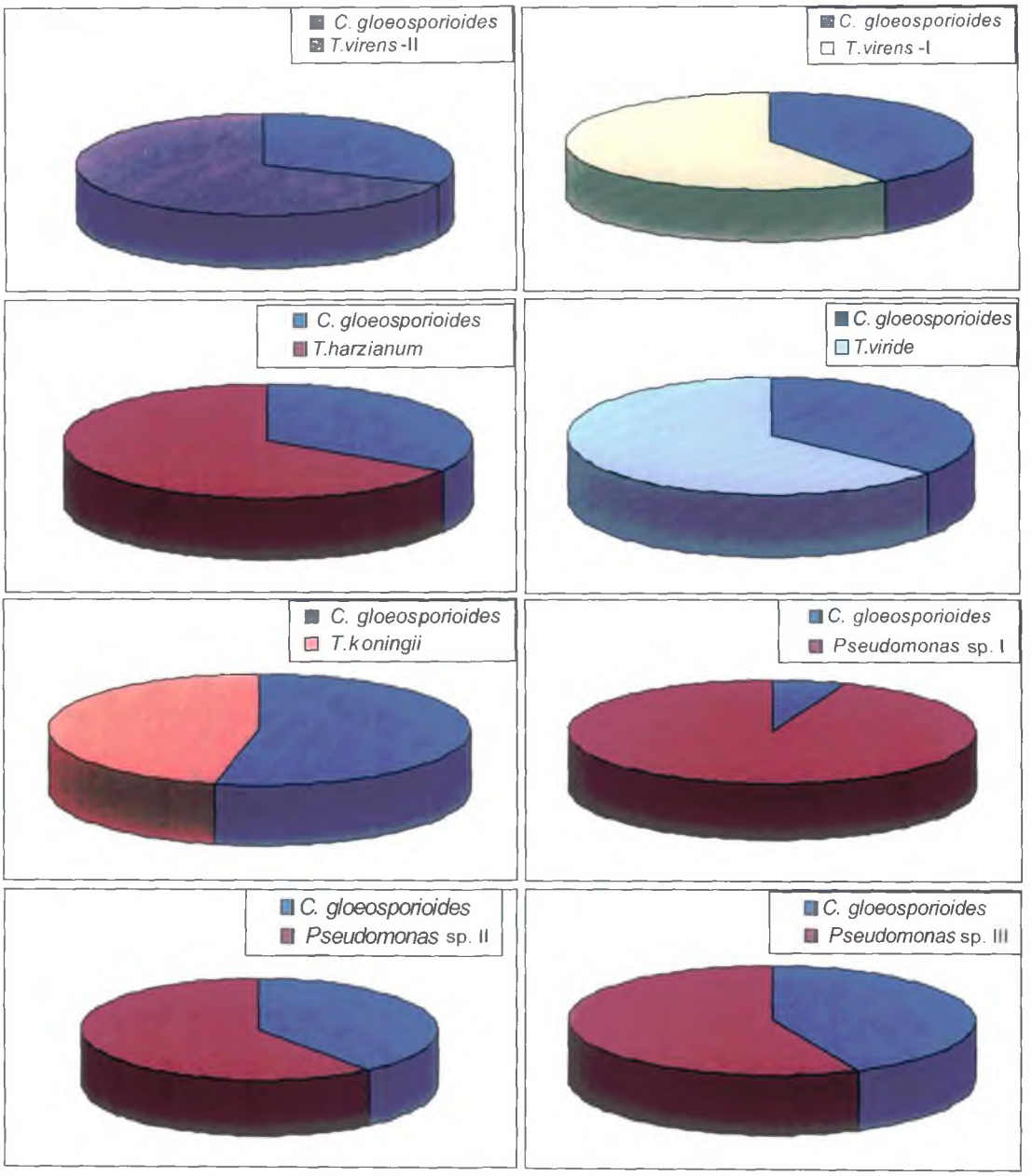

Fig. 21 : Effect of antagonist on the growth of *Colletotrichum gloeosporioides.*

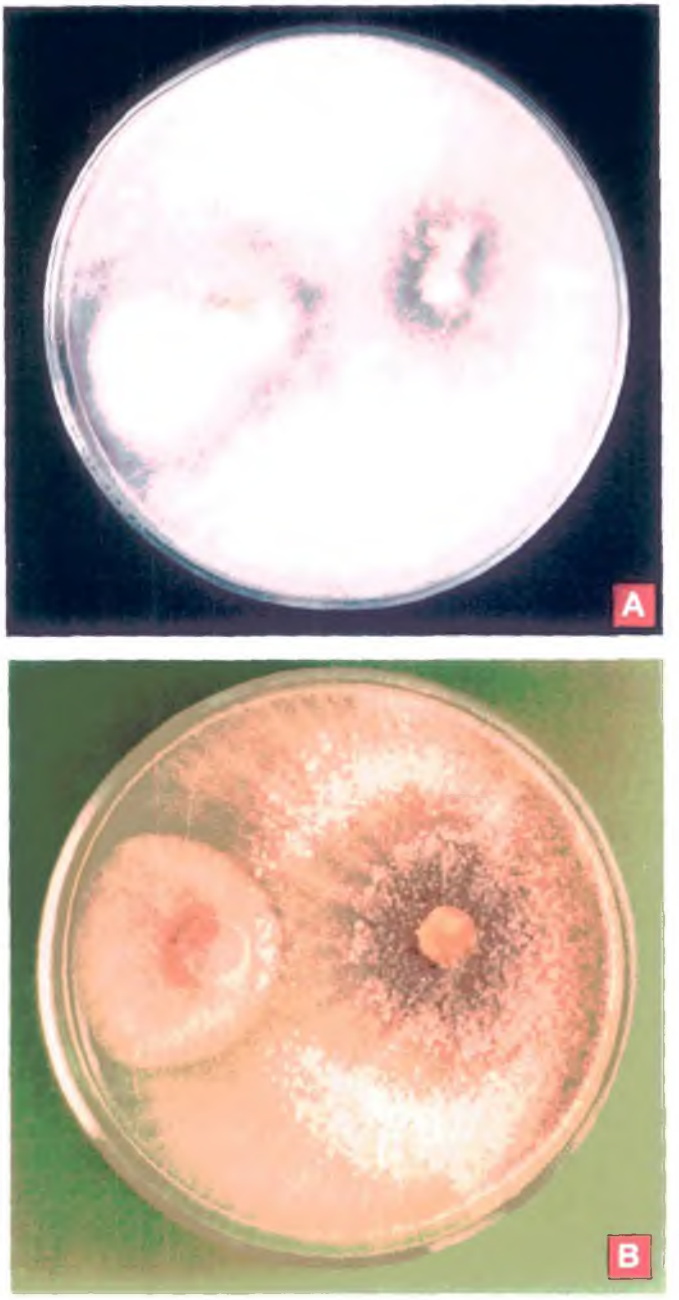

Plate XV : Biocontrol by antagonistic microorganisms.

Fig. A : Dual culture of *Trichoderma viride* and *Colletotrichum gloeosporioides*.

Fig. B : Dual culture of *Trichoderma harzianum* and *C. gloeosporioides*.

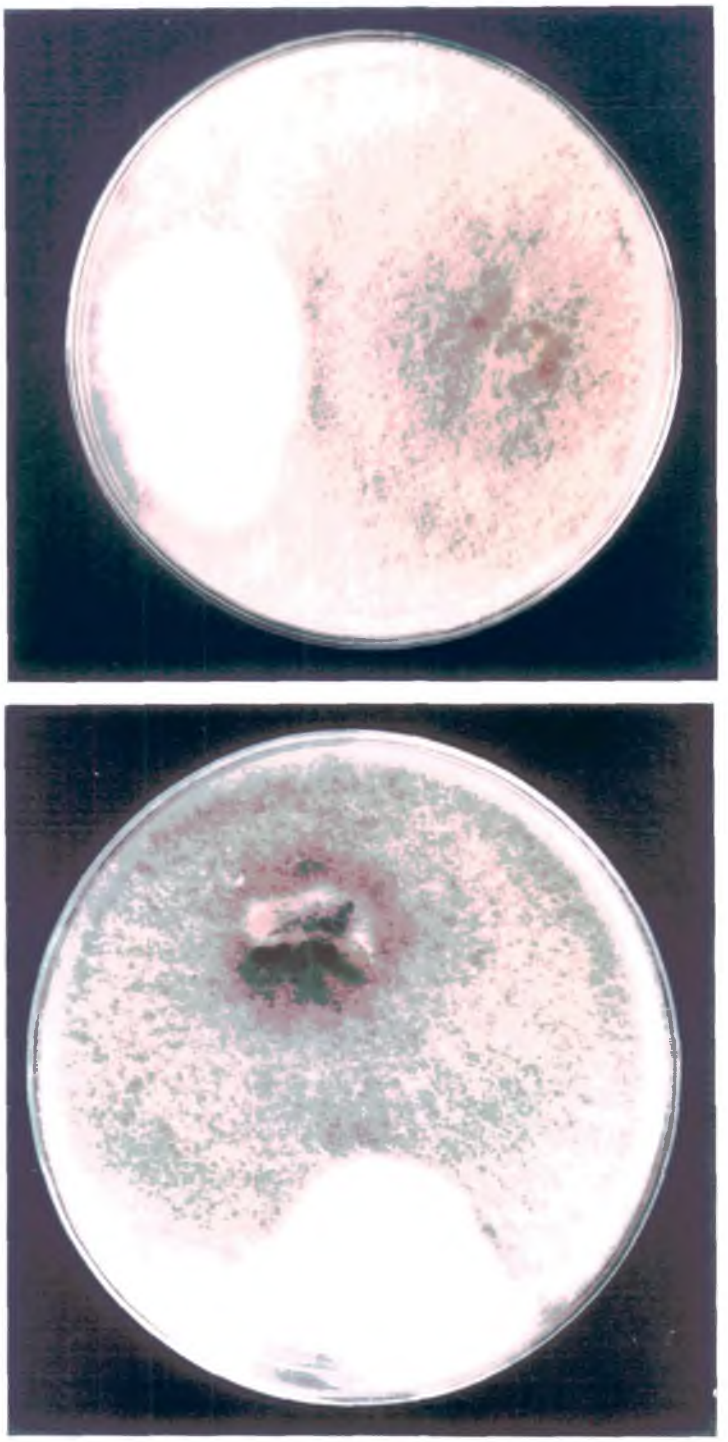

Plate XVI : Biocontrol by antagonistic microorganisms.

Fig. A : Dual culture of *Trichoderma virens* (Isolate-I) and *C. gloeosporioides.*

Fig. B : Dual culture of *Trichoderma virens* (Isolate-II) and *C. gloeosporioides.*

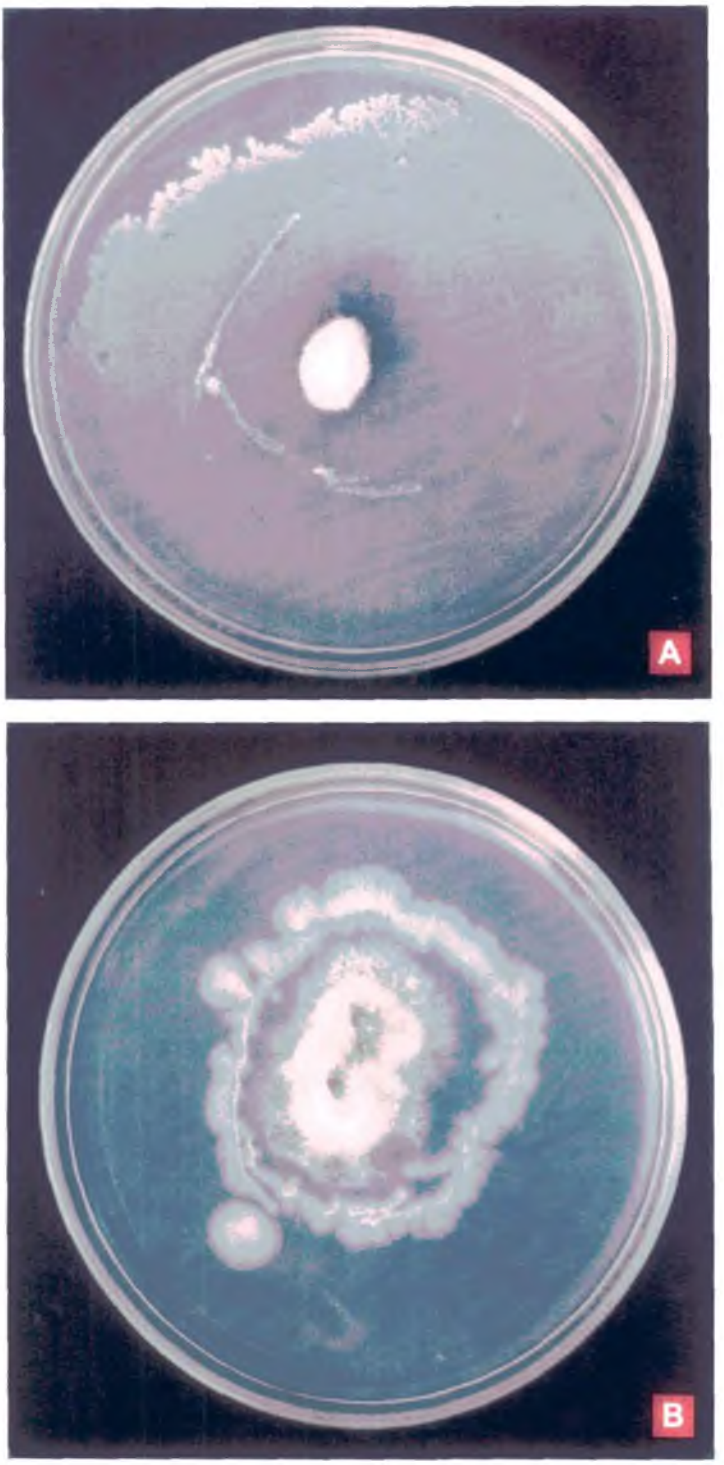

Plate XVII : Biocontrol by antagonistic microorganisms.

Fig. A : Dual culture of *Pseudomonas* sp. (Isolate-I) and *C. gloeosporioides.*

Fig. B : Dual culture of *Pseudomonas* sp. (Isolate-II) and *C. gloeosporioides.*

Table 28. *In vitro* effect of cell free culture filtrates of fungal antagonists on the growth of *C. gloeosporioides.*

Antagonists	Radial growth (mm)* of *C. gloeosporioides* in PDA*** after 5 days of inoculation	Percent inhibition over control**
Trichoderma viride	38.00±1.26	45.71±1.80
T. koningii	59.83±0.93	14.53±1.32
T. harzianum	32.50±0.76	53.57±1.09
T. virens (Isolate I)	39.00±1.53	44.29±2.18
T. virens (Isolate II)	30.83±1.01	55.96±1.45
CD at 5%	2.86	4.08

* Mean of three replications. Data after ± represent standard error values.
**Control diameter = 70 mm after 5 days of inoculation.
*** PDA : Culture filtrate = 9:1.

In experiments where crude culture filtrates were evaluated, results (Table 29) showed that four of the five antagonists, *T. viride, T. harzianum, T. virens* (Isolate-I) and *T. virens* (Isolate-II) totally checked the growth of the pathogen *C. gloeosporioides. T. koningii* showed an inhibition of 70.96% (Plate XVIII).

Table 29. *In vitro* effect of fungal antagonists on the growth of *C. gloeosporioides* crude culture filtrates.

Antagonists	Radial growth (mm)* of *C. gloeosporioides* in PDA*** after 5 days of inoculation	Percent inhibition over control**
Trichoderma viride	0	100
T. koningii	20.33±0.67	70.96±o.98
T. harzianum	0	100
T. virens (Isolate I)	0	100
T. virens (Isolate II)	0	100
CD at 5%	0.75	1.10

* Mean of three replications. Data after ± represent standard error values.
**Control diameter = 70 mm after 5 days of inoculation.
*** PDA : Culture filtrate = 9:1.

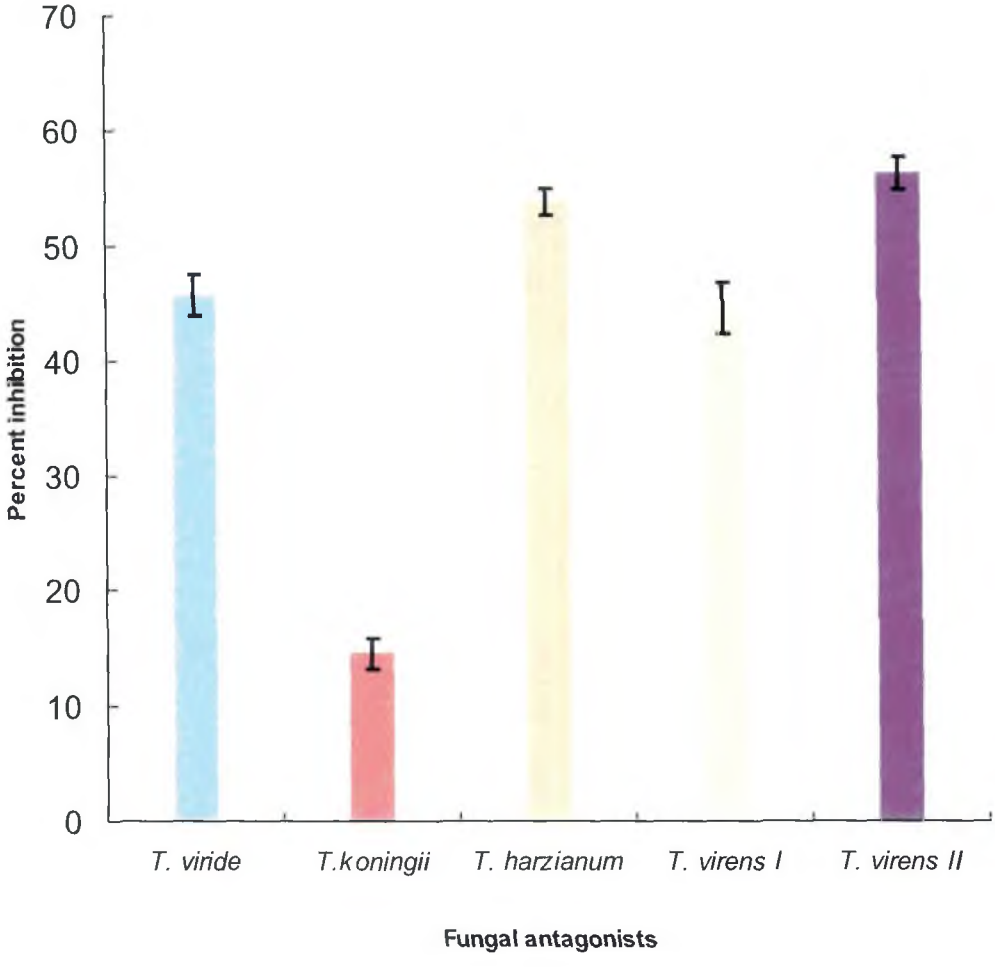

Fig. 22 : *In vitro* effect of cell free culture filtrates of fungal antagonists on the growth of *C. gloeosporioides* in PDA after 5 days of incubation.

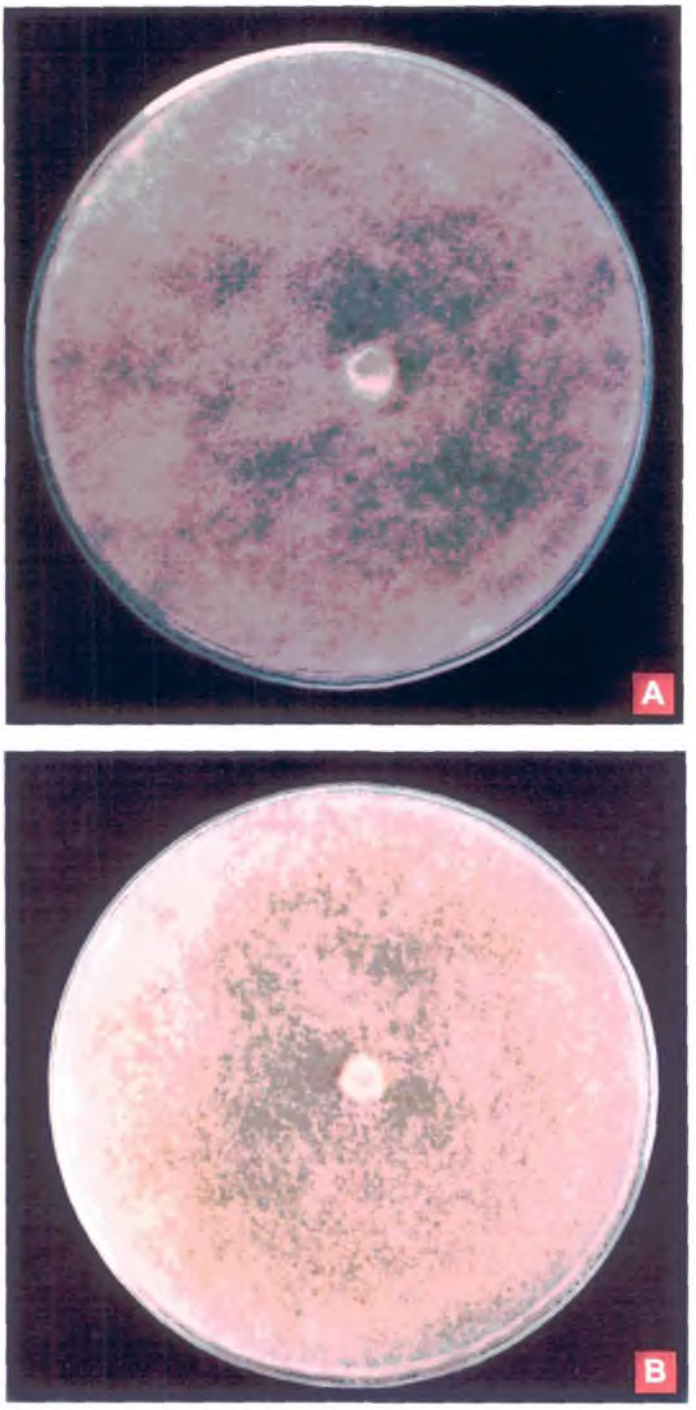

Plate XVIII : Biocontrol by antagonistic microorganisms.

Fig. A : Control of *C. gloeosporioides* by crude culture filtrate containing spores of *Trichoderma virens* (Isolate-II).

Fig. B : Control of *C. gloeosporioides* by crude culture filtrate containing spores of *Trichoderma harzianum.*

4.7.2. *In vivo* effect of crude culture filtrate of fungal antagonists

Antagonistic activity of culture filtrates of *T. viride, T. koningii, T. harzianum, T. virens* (Isolate-I) and *T. virens* (Isolate-II) against *C. gloeosporioides* were tested in susceptible variety of brinjal plant (Pusa purple long) which were sprayed with crude culture filtrates that contained spores of the antagonists in separate sets (10 plants/set). The plants in each set were inoculated with *C. gloeosporioides* following the technique as described in materials and methods (Section 3.17.3.). The whole experiment was repeated thrice. Mean disease index / plant was calculated after 3, 6, 9, and 12 days of inoculation following the procedure as described by Sinha and Das (1972) and was tabulated in Table 30 and graphically represented in Fig. 23. From the result it was clear that crude culture filtrates of *Trichoderma virens* (Isolate-II) showed maximum reduction of disease with mean disease index of 4.63 followed by *T. harzianum* with mean disease index of 6.73. Other two antagonists *T. viride* and *T. virens* (Isolate-I) also showed significant control of the disease.

Table 30. *In vivo* control of anthracnose of brinjal (Pusa purple long) caused by *C. gloeosporioides* by foliar treatment of crude culture filtrate of some fungal antagonists.

Antagonist	Mean disease index / plant* Incubation periods (Days)			
	3	6	9	12
T. viride	1.23±0.15	5.17±0.37	7.13±0.50	7.73±0.67
T. harzianum	0.97±0.18	3.90±0.47	5.37±0.66	6.73±0.46
T. virens (Isolate-I)	1.25±0.16	4.57±0.37	6.37±0.38	8.20±0.60
T. virens (Isolate-II).	0.98±0.16	3.57±0.30	4.26±0.41	4.63±0.35
Control	2.30±0.36	6.97±0.27	12.43±0.94	17.43±0.97
CD at 5%	0.44	1.00	1.66	1.33

* Mean of three replications. Data after ± represent standard error values.

4.8. Screening of potential antifungal properties from different plant extracts against *C. gloeosporioides*

In an approach to further develop a complete ecofriendly method for controlling anthracnose of brinjal, botanical extracts were tested for their antifungal properties. Several workers have successfully utilized extracts of numerous plants for

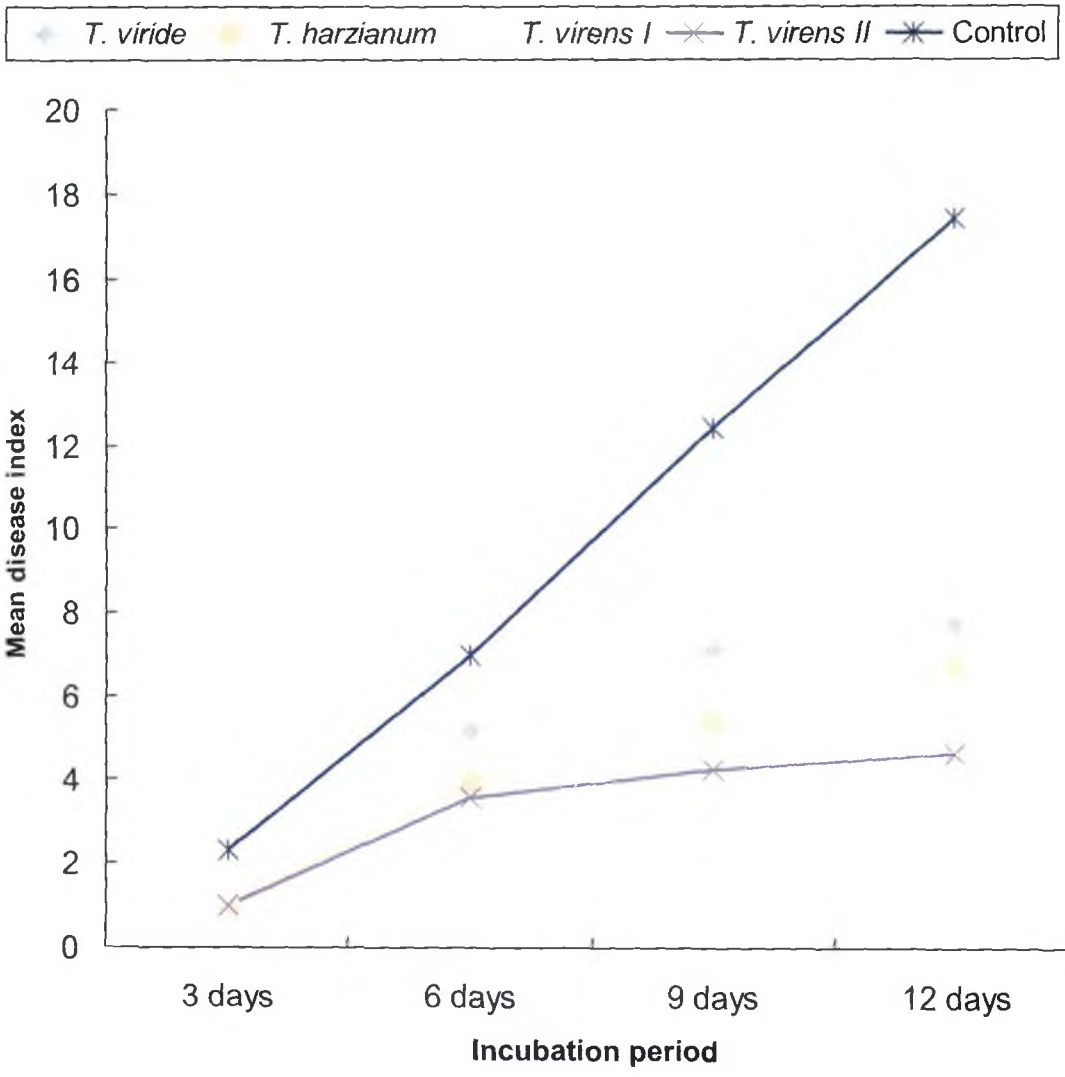

Fig. 23 : Graphical representation of control of anthracnose of brinjal (Pusa purple long) caused by *C. gloeosporioides* by foliar treatment of crude culture filtrate of some fungal antagonists.

controlling plant diseases. Hence, several plants were collected from different parts of sub Himalayan West Bengal and study was undertaken to screen the potential antifungal activity against the pathogen.

4.8.1. Slide germination bioassay to screen potential botanicals for controlling *C. gloeosporioides*

To screen potential antifungal activity 84 plants were collected from different parts of sub Himalayan West Bengal. Extracts (both aqueous and 50% ethanolic) of the plant parts (leaves, bulbs, rhizomes as applicable) were prepared and used for screening their antifungal properties against *C. gloeosporioides*. Slide germination technique described by Suleman *et al.* (2002) was followed for the screening. Details of the procedure of the slide germination bioassay and the preparation of both the aqueous and 50% ethanol extracts of the plant parts has been discussed in the materials and methods (Section 3.18.1 and 3.18.2). On the basis of the percentage of spore germination, those species which produced less than 60% germination of spores in atleast one of the extracts were selected and altogether 23 plants were short listed. The percent germination and inhibition of spore germination against the respective plant extracts as well as the average germ tube length were calculated. Antifungal activity of these plants are presented in the Table 31.

Both the aqueous and 50% ethanol extracts of *Datura metei, Cannabis sativa* and *Allium sativum* showed 100 percent inhibition of spore germination. Aqueous extract of *Polyalthia longifolia* showed 90.07% inhibition the while 50% ethanol extract showed 80.07% inhibition of spore germination. Other aqueous extracts that showed 80% inhibition of spore germination were *Solanum torvum* (80.50%), *Aegle marmelos* (84.17%), *Solanum khasianum* (88.83%) and *Melia dubia* (82.76%). Among the other 50% ethanol extracts, *Aegle marmelos* (89.17%) and *Vitex negando* (80.50%) showed high percentage of inhibition. Other species whose extracts were found to have significant antifungal activity were *Murraya koenigii, Psidium guajava, Solanum xanthocarpum, Dioscorea alata, Zingiber officinale* (all aqueous extracts) and *Syzygium cumini, Piper peepuloides* and *Azadirachta indica* (50% ethanol extracts).

Table 31. Effect of different plant extracts on spore germination of *C. gloeosporioides* after 48 h of incubation.

Plant species tested (Family of the plant)	Aqueous extract			50% ethanol extract		
	Percent* germination	Percent* inhibition	Average* germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Aegle marmelos (L.) Corr. (Rutaceae)	15.83 ±0.93	84.17 ±0.93	24.83 ±0.93	10.83 ±1.09	89.17 ±1.09	21.83 ±0.93
Allium sativum L. (Alliaceae)	0	100	0	0	100	0
Azadirachta indica A. Juss. (Meliaceae)	43.70 ±1.39	56.30 ±1.39	42.50 ±1.26	32.67 ±1.09	67.33 ±1.09	35.33 ±1.45
Cannabis sativa L. (Cannabinaceae)	0	100	0	0	100	0
Citrus limon Burm. f. (Rutaceae)	74.33 ±1.86	25.67 ±1.86	67.5 ±1.04	44.83 ±1.96	55.17 ±1.96	54.83 ±1.17
Datura metel L. (Solanaceae)	0	100	0	0	100	0
Dioscorea alata L. (Dioscoriaceae)	37.17 ±1.96	62.83 ±1.96	54.67 ±2.19	100	0	86.50 ±2.75
Dryopteris filix-mas (L.) Schott (Polypodiaceae)	100	0	94.00 ±1.53	64.33 ±2.73	35.67 ±2.73	64.83 ±1.64
Melia dubia Cav. (Meliaceae)	17.67 ±0.92	82.76 ±0.60	23.50 ±1.04	33.67 ±1.48	66.33 ±1.48	25.33 ±1.20
Murraya koenigii (L) Spreng (Rutaceae)	28.66 ±1.17	71.33 ±1.17	43.83 ±1.88	65.67 ±2.40	34.33 ±2.40	66.50 ±1.26
Piper peepuloides Roxb (Piperaceae)	42.33 ±1.09	57.67 ±1.09	54.00 ±1.53	37.83 ±1.36	62.17 ±1.36	32.33 ±1.76
Polyalthia longifolia (Sonnerat) Thwaites (Annonaceae)	9.93 ±0.47	90.07 ±0.47	35.83 ±1.69	19.93 ±1.16	80.07 ±1.16	21.83 ±2.05
Psidium guajava L. (Myrtaceae)	32.57 ±0.81	67.43 ±0.81	44.67 ±2.33	55.33 ±1.86	44.67 ±1.86	55.67 ±1.20

Contd... Table 31

Table 31 (Contd.....)

Effect of different plant extracts on spore germination of *C. gloeosporioides* after 48 h of incubation.

Plant species tested (Family of the plant)	Aqueous extract			50% ethanol extract		
	Percent* germination	Percent* inhibition	Average* germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Smilax zeylanica L. (Smilaceae)	64.67 ±1.86	35.33 ±1.86	62.33 ±1.45	44.00 ±1.32	56.00 ±1.32	32.83 ±1.59
Solanum khasianum Clarke (Solanaceae)	11.17 ±0.68	88.83 ±0.68	26.17 ±1.09	34.17 ±1.01	65.83 ±1.01	41.83 ±1.30
Solanum torvum Sw. (Solanaceae)	19.50 ±1.26	80.50 ±1.26	37.50 ±1.32	29.07 ±1.34	70.93 ±1.34	24.17 ±1.09
Solanum xanthocarpum Schard & Wendl. (Solanaceae)	36.50 ±1.76	63.50 ±1.76	38.83 ±1.64	43.17 ±1.59	56.83 ±1.59	37.67 ±1.01
Strobilanthes cusia O. Kuntze. (Acanthaceae)	69.83 ±1.69	30.17 ±1.69	59.33 ±2.85	56.00 ±1.32	44.00 ±1.32	47.17 ±1.58
Syzygium cumini (L.) Skeels (Myrtaceae)	45.17 ±1.09	54.83 ±1.09	35.83 ±0.93	34.67 ±1.96	65.67 ±1.64	31.83 ±1.42
Vitex negando L. (Verbenaceae)	32.17 ±0.93	67.83 ±0.93	28.16 ±1.42	19.50 ±0.76	80.50 ±0.76	28.33 ±0.93
Zingiber capitatum Roxb. (Zingiberaceae)	55.17 ±1.59	44.83 ±1.59	55.50 ±0.87	52.67 ±1.09	47.33 ±1.09	44.67 ±2.19
Zingiber chrysanthum Roscoe (Zingiberaceae)	43.33 ±1.69	55.67 ±1.69	42.33 ±1.20	45.63 ±0.68	54.37 ±0.68	35.83 ±0.93
Zingiber officinale Roscoe (Zingiberaceae)	38.83 ±1.36	61.17 ±1.36	37.83 ±2.13	45.17 ±2.24	54.83 ±2.24	46.83 ±1.48
Control	100.00	0	103.83 ±2.24	100.00	0	97.83 ±2.62
C.D. at 5%	0.92	0.92	1.36	1.40	1.38	1.00

*Mean of 3 replications. Data after ± represent standard error values.

When the spores were allowed to germinate in the presence of these extracts, average germ tube length was found less in comparison to control. Maximum inhibition in the germ tube length in comparison to control (103.83 µm

and 97.83 µm for aqueous and 50% ethanol extract respectively) was found in 50% ethanolic extract of *Polyalthia longifolia* (21.83 µm) followed by aqueous extract of *P. longifolia* (35.83 µm). Effective extracts obtained from Table 31 were selected for agar cup bioassay and further for TLC plate bioassay to detect the presence of specific antifungal principles.

4.8.2. Agar cup method for detection of antifungal activity of selected plant extracts on the growth of *C. gloeosporioides*

Eleven plant extracts were tested following agar cup bioassay technique (Section 3.18.3) to test their antifungal activity. Radial measurement of inhibition zones of different plant extract was tabulated in Table 32. From the result it was clear that *Allium sativum* was most inhibitory which developed a large inhibition zone with the diameter of 40 mm. *Datura metel* and *Polyalthia longifolia* showed inhibition zones of 18.17 mm and 13.20 mm respectively. Least inhibition zone was produced by *A. indica* (8.33 mm).

Table 32. Effect of antifungal activity of selected plant extracts on the growth of *C. gloeosporioides* tested by the agar cup method.

Plant extracts	Plant parts used	Radial measure of inhibition zone (mm)*
Aegle marmelos	Leaf	10.03±0.44
Allium sativum	Bulb	40.00±1.53
Azadirachta indica	Leaf	08.33±0.58
Cannabis sativa	Leaf	08.63±0.72
Datura metel	Leaf	18.17±0.60
Melia dubia	Leaf	09.30±0.53
Polyalthia longipholia	Leaf	13.20±1.35
Psidium guajava	Leaf	12.40±0.95
Vitex negando	Leaf	11.23±0.73
Zingiber officinale	Rhizome	10.23±0.87
Zingiber chrysanthum	Rhizome	09.33±0.55
CD at 5%		1.37

* Mean of three replications. Data after ± represent standard error values.

4.8.3. TLC plate bioassay for detection of antifungal properties of selected plant extracts

The selected extracts were run on previously activated TLC plates and the plates were developed in the solvent, chloroform: methanol (9:1) and dried in the air at room temperature (30 °C). Spores of *C. gloeosporioides* were mixed with Richard's solution and were sprayed on the developed plates by an automizer. The plates were placed in a humid chamber at 25±1 °C for 2-3 days. Details of the techniques have been presented under materials and methods (Section 3.18.4).

After 3 days, the inhibition zones were observed and the diameter of the inhibition zones were measured. The R_f of the inhibition zones were also calculated and have been presented in Table 33. *D. metel* showed three distinct antifungal zones (Plate XIX b1, b2 & b3). R_f of b1 was 0.25 and had a diameter of 9 mm. Diameter of b2 was 19 mm with the R_f of 0.65. The R_f and diameter of b3 was 0.97 and 10 mm respectively. *P. longifolia* developed two antifungal zones (Plate XIX a1 & a2). The diameter and R_f of a1 was 21 mm and 0.33 respectively. The diameter of a2 was 8 mm and R_f was 0.96. Bulb extracts of *A. sativum* showed a large inhibition zone (Plate XIX - d1) of 24 mm diameter and with an R_f of 0.95. *C. sativa* developed two inhibition zones (Plate XIX c1 & c2). They had the R_f of 0.17 and 0.23 and the diameter of 17 mm and 11 mm respectively. Similarly *A. indica* showed two small inhibition zones of 0.5 mm each with the R_f of 0.48 and 0.67 respectively.

From the result obtained in TLC plate bioassay, it was evident that five plant extracts showed inhibition zones on TLC plates. They were *D. metel, A. sativum, P. longifolia, C. sativa* and *A. indica*. Therefore those five plants were selected for further *in vitro* test following poisoned food technique and for *in vivo* application on infected plants of a susceptible brinjal variety (Pusa purple long) to test their antagonistic potential as botanicals against anthracnose.

Table 33. TLC plate Bioassay of extracts of five different plants against *Colletotrichum gloeosporioides.* .

Plant extracts*	Inhibition zone**		
	Diameter (mm)		R_f
Polyalthia longifolia	a1	21	0.33
	a2	08	0.96
Datura metel	b1	09	0.25
	b2	19	0.65
	b3	10	0.97
Cannabis sativa	c1	17	0.17
	c2	11	0.23
Allium sativum	d1	24	0.95
Azadirachta indica	-	05	0.48
	-	05	0.67

* 50% ethanol extracts used.; ** Solvent used chloroform: methanol = 9:1.

4.8.4. Antifungal effect of selected plant extracts on growth of *C. gloeosporioides* following poisoned food technique

Poisoned food technique was performed with five selected aqueous plant extracts as described in the materials and methods (Section 3.18.5). These were *D. metel* (Plate XXB), *A. sativum* (Plate XXC), *P. longifolia* (Plate XXA), *C. sativa* (Plate XXD) and *A. indica.* Results of the experiments performed in two different media viz. PDA and antifungal assay agar are tabulated in Table 34 and Table 35 respectively. *A. sativum* bulb extracts totally checked the growth of the pathogen *C. gloeosporioides* in PDA (Plate XXIB). Other extracts such as *D. metel* (Plate XXI A), *P. longifolia* (Plate XXID) and *Azadirachta indica* showed 73.89%, 52.59% and 45.56% growth inhibition respectively (Fig. 24). In antifungal assay agar (Table 35), *A. sativum* bulb extracts was again found to produce 100% inhibition over control on the radial mycelial growth of *C. gloeosporioides. P. longifolia* and *D. metel* leaf extracts showed growth inhibition of 49.53% and 36.19% respectively.*C. sativa* (Plate XXIC) produced least inhibition among the five tested botanicals in both media.

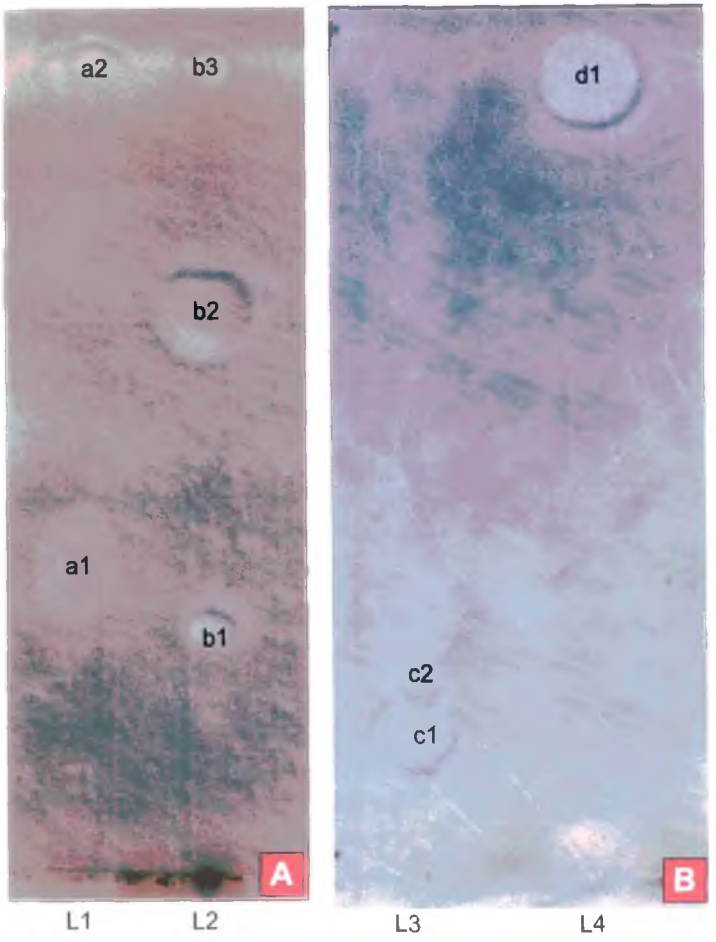

Plate XIX : TLC- Plate bioassay.

Fig. A : L1 - *Polialthia longifolia* (a1 & a2),
L2- *Datura metel* (b1, b2 & b3).

Fig. B : L3- *Cannabis sativa* (c1 &c2),
L4- *Allium sativum* (d1).

Plate XX

Fig. A : Twig of *Polyalthia longifolia* **Fig. B :** Twig of *Datura metel*

Fig. C : Bulb of *Allium sativum* **Fig. D :** Twig of *Cannabis sativa.*

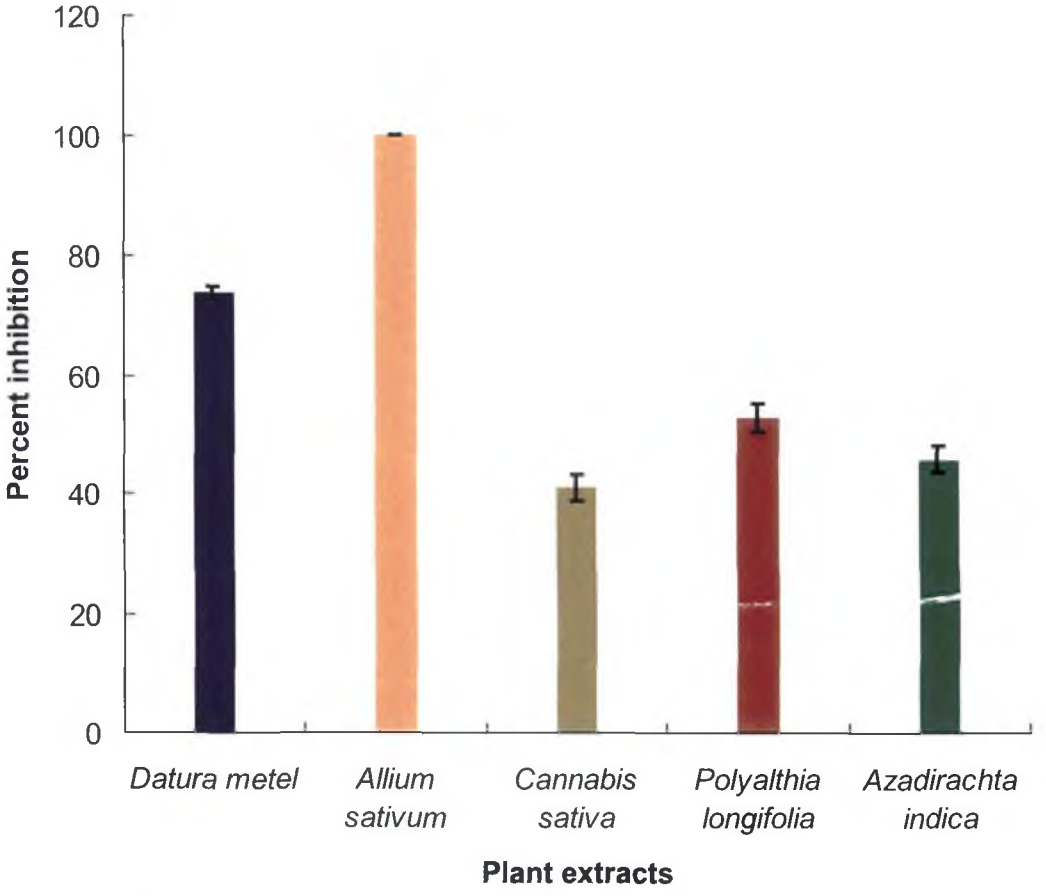

Fig. 24 : Graphical representation of antifungal effect of some plant extracts on the growth of *C. gloeosporioides* in PDA.

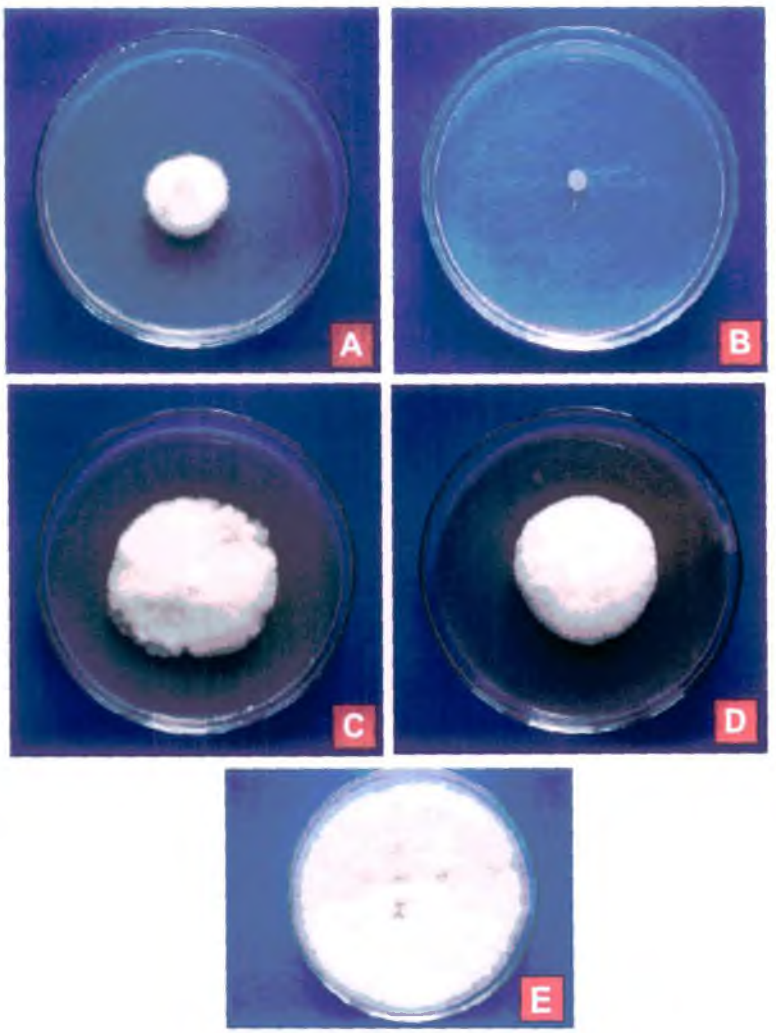

Plate XXI : Growth of *Colletotrichum gloeosporioides* in PDA medium using different plant extracts following Poisoned food technique.

Fig. A : *Datura metel* leaf extract,

Fig. B : *Allium sativum* bulb extract,

Fig. C : *Cannabis sativa* leaf extract,

Fig. D : *Polyalthia longifolia* leaf extract,

Fig. E : Control (without any leaf extract).

Table 34. Antifungal effect of selected plant extracts on the growth of *C. gloeosporioides* in PDA.***

Plant extracts	Radial growth (mm)* of *C. gloeosporioides* after 7 days of inoculation	Percent inhibition over control**
Allium sativum	0	100
Azadirachta indica	49.00±2.08	45.56±2.31
Cannabis sativa	53.17±1.92	40.93±2.13
Datura metel	23.50±0.87	73.89±0.96
Polyalthia longifolia	42.67±2.19	52.59±2.43
CD at 5%	2.50	2.78

*Control diameter = 90 mm; ** Mean of three replications; *** PDA : extract = 9:1.

Table 35. Antifungal effect of selected plant extracts on the growth of *C. gloeosporioides* in antifungal assay agar. ***

Plant extracts	Radial growth (mm)* of *C. gloeosporioides* after 7 days of inoculation	Percent inhibition over control**
Allium sativum	0	100
Azadirachta indica	24.50±0.76	30.00±2.18
Cannabis sativa	25.33±0.88	27.62±2.52
Datura metel	22.33±0.93	36.19±2.65
Polyalthia longifolia	17.67±0.93	49.53±2.65
CD at 5%	2.20	6.29

*Control diameter = 35 mm; ** Mean of three replications;*** Media :extract = 9:1.

4.8.5. Foliar application of plant extracts for controlling anthracnose caused by *C. gloeosporioides* in brinjal plants

Antagonistic activity of aqueous extracts (2 g in 10 ml distilled water) of *Datura metel, Allium sativum, Polyalthia longifolia, Cannabis sativa* and *Azadirachta indica* were tested *in vivo* on a susceptible brinjal variety (Pusa purple long). The plants were sprayed with crude aqueous extracts in separate sets. On control set, sterile distilled water was sprayed. After 24 hours, the plants in each set were inoculated with *C. gloeosporioides* following the methods as described under materials and methods (Section 3.18.6.). In control set, the plants were inoculated

with spore suspension of *C. gloeosporioides* without any extract spraying. Following the procedure of Sinha and Das (1972) mean disease index / plant was calculated after 3, 6, 9 and 12 days of inoculation to compare with the control set. The results were noted in the Table 36 and also graphically represented in Fig. 25.

Table 36. *In vivo* control of anthracnose of brinjal (Pusa purple long) caused by *C. gloeosporioides* by foliar treatment with crude plant extracts.

Plant species tested	Mean disease index / plant*			
	Incubation periods (Days)			
	3	6	9	12
Allium sativum	0	0.70±0.12	1.43±0.12	2.67±0.19
Azadirachta indica	1.63±0.18	2.93±0.27	4.07±0.39	4.73±0.27
Cannabis sativa	0.83±0.19	2.43±0.19	3.40±0.17	4.53±0.19
Datura metel	0	1.67±0.12	2.70±0.31	3.43±0.23
Polyalthia longifolia	1.37±0.26	2.53±0.27	4.00±0.25	4.80±0.23
Control	2.30±0.36	6.97±0.27	12.43±0.94	17.43±0.97
CD at 5%	0.33	0.50	1.10	0.96

* Mean of three replications. Data after ± represent standard error values.

From the result it was evident that all the five extracts showed significant reduction in mean disease index. *A. sativum* bulb extracts showed maximum control of the disease with mean disease index of only 2.67 followed by *D. metel* with mean disease index of 3.43 after 12 d of inoculation. *P. longifolia, C. sativa* and *A. indica* showed mean disease index of 4.80, 4.53 and 4.73 respectively. In control set, disease occurrence was always found to be higher than the experimental sets.

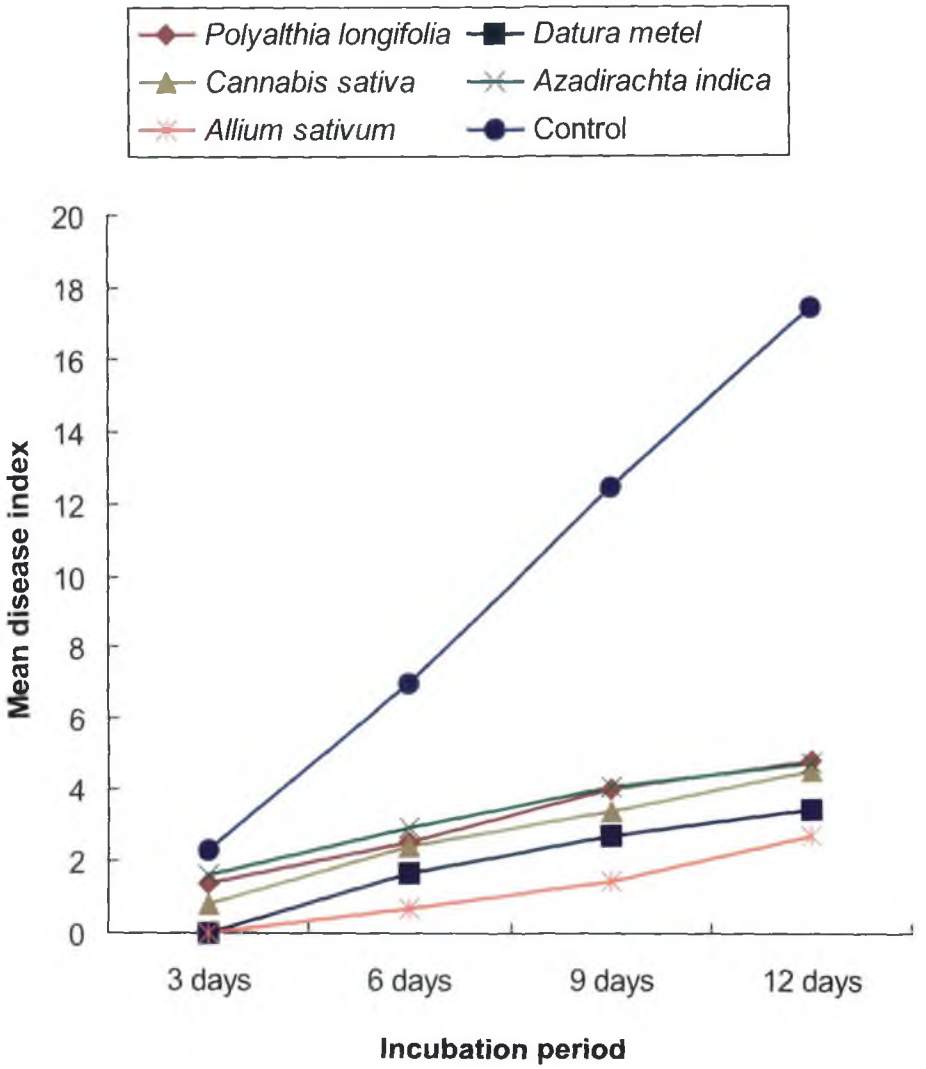

Fig. 25 : Graphical representation of control of anthracnose of brinjal (Pusa purple long) caused by *C. gloeosporioides* by foliar treatment with crude plant extracts.