CHAPTER II

4.2 : Chapter II : *Alternaria alternata*, a pathogen of tomato (*Lycopersicum esculentum*) and its control.

Study of *A. alternata*: The morphology of the pathogen *A. alternata* was observed in PDA. The morphology of the fungus was studied in light microscope. For microscopic observation, 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. Immature mycelia were hyaline in colour but on maturity it became gray in colour. Conidia of the fungus were obclavate to beaked and brownish to olive in colour having transverse and longitudinal septa (Plate-28, fig. b & c). Conidia were produced from simple septate conidiophores in simple or branched acropetal chains. The length and breadth of mature conidia were 18-36 µm and 5-10 µm respectively. The diameter of the mature hyphae ranged between 3-5 µm. The scanning electron microscopic studies were also performed to understand the details of the suface morphology of the fungal pathogen as well as to get accurate measurement of the spore and hyphae. The measurements have been shown directly on the electron microscopic photographs presented in plate-29.

4.2.1 : Pathogenicity test of *Alternaria alternata* in different tomato varieties.

Whole plant inoculation technique was followed to determine pathogenicity of *Alternaria alternata,* a pathogen of tomato plant. Seven tomato varieties (viz. Karan, Romeo, Pasuja, Trishul, Mahyco, Abinash-2 and US1080) were selected for the experiment. The details of the experiment and disease assessment procedures have been mentioned in the materials methods (section 3.7). From the data (represented in the table-4.2.1.) it is quite clear that Karan variety showed maximum disease development (mean disease index /plant was 6.8 after 10 days of inoculation) against *A.alternata.* Therefore tomato plants of karan variety were considered as the most susceptible variety towards the pathogen among the varieties tested. Varieties like romeo, pasuja, Trishul, mahyco and Abinash-2 were also considered as susceptible as their mean disease index/plant were 5.0, 3.7 3.6, 0.6 and 0.5 respectively after 10 days of inoculation. On the other hand US1080 variety was considered as the most resistant as it showed no disease symptoms.

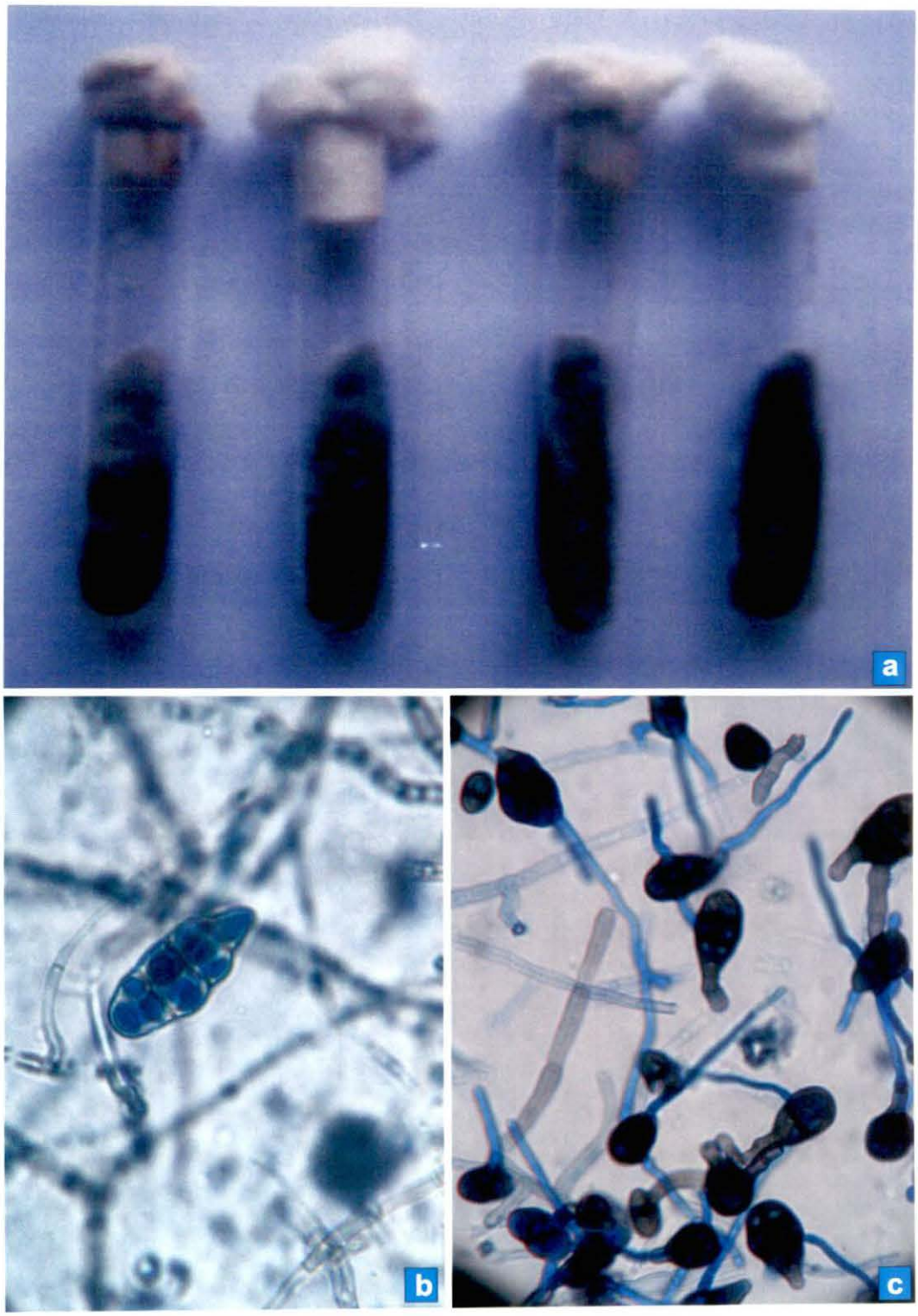

Plate 28: fig. a. Mycelial growth of *Alternaria alternata* in PDA slants
fig.b. Spore of *Alternaria alternata* showing distinct septa
fig. c. Germinating spores of *Alternaria alternata*.

Table: 4.2.1 : Pathogenicity of *Alternaria alternata* on tomato varieties following whole plant inoculation technique

Tomato varieties	Incubation period (Days)				
	2	4	6	8	10
	MDI/ plant*	MDI/ plant	MDI/ plant	MDI/ plant	MDI/ plant
Karan	0	1.7	3.1	4.9	6.8
Romeo	0	1.3	2.4	3.9	5.0
Pasuja		0	1.8	2.4	3.7
Trishul	0	0	0.9	2.1	3.6
Mahyco	0	0	0	0.4	0.6
Avinash 2	0	0	0	0.3	0.5
US1080	0	0	0	0	0
CD at 5%		0.89	0.19	0.14	0.13

MDI= Mean disease index; MNL =Mean number of lesions

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

** Average of 50 lesions.

4.2.2 : Screening of potential antifungal activity against *Alternaria alternata* (following spore germination bioassay on sterilized glass slides)

All the eighty (80) plants screened for antifungal activity against the pathogens of eggplants were also used for screening of antifungal activity against *A. alternata,* a pathogen of tomato. Extracts of the leaves (50% ethanolic) were prepared and were used for their antifungal properties against *A. alternata.* A detailed procedure of spore germination bioassay and preparation of extracts (50% ethanolic) have been described in materials and methods (section 3.10). Data presented in the table were calculated on the basis of the percent inhibition of spore germination of the fungi in different plant extracts tested. It was evident from the result (Table-4.2.2) that 50% ethanolic extract of *D. innoxia, D. metel, X. strumarium* and *D. stramonium* were effective in controlling spore germination of the fungal pathogen completely. The percent inhibition of the plant extracts have been presented in table- 4.2.2. Inhibition percentage of each of the plant extracts (against *A. alternata*) have been shown in the following lines. About 98% inhibition was recorded by *Clerodendrum viscosum.* Four plant extracts (of *Allium sativum, Nyctanthes arbortristis, Annona squamosa* and *Plumeria rubra)* inhibited spore germination within the range of 90 - 95%. Eight plant extracts (*Azadirachta indica, Borreria alata, Clausena excavata, Eucalyptus globosus, Melia dubia, Psidium guajava, Ocimum gratissimum* and *Polyalthia longifolia*) inhibited spore germination within the range of 80 - 90%.

Table 4.2.2: Effect of different plant extracts on spore germination of *Alternaria alternata*

Name of plants	Family	*Alternaria alternata* (50 % alcoholic extract)	
		% Germination	% Inhibition
Acacia catechu (L.f) Wild	Mimosaceae	67	33
Acalypha indica L.	Euhorbiaceae	25	75
Adhatoda vasika Nees.	Acanthaceae	68	32
Aegle mermelos (L.) Corr	Rutaceae	72	28
Ageratum conzoides L.	Asteraceae	30	70
Allium sativum L.	Liliaceae	10	90
Alostonia scholaris (L.) R. Br.	Apocynanceae	52	48
Amaranthus spinosus L.	Amaranthaceae	98	02
Anisomeles indica (L.) Kuntze	Laminaceae	55	45
Annona squamosa L.	Annonaceae	10	90
Argemone mexicana L.	Papavaraceae	68	32
Artocarpus heterophylus Lam.	Moraceae	70	30
Asparagus racemosus Wild	Asparagaceae	37	63
Azadirachta indica L.	Melicaceae	20	80
Borreria alata (Aublet) De Candolle.	Rubiaceae	12	88
Caesalpinia pulcherrima (L.) Swartz.	Caesalpiniaceae	74	26
Cannabis sativa L.	Cannabinaceae	68	32
Calotropis gigantea (L.) R.Br.ex Aiton	Asclepiadaceae	93	7
Cassia tora L.	Caesalpiniaceae	85	15
Catharanthus roseus L. Don	Apocynanceae	68	32
Centella asiatica L.	Apiaceae	70	30
Citrus limon (L.) Burm.	Rutaceae	90	10
Clerodendrum viscosum Vent.	Verbenaceae	02	98
Clausena excavata Burm.f.	Rutaceae	11	89
Clitoria ternatea L.	Fabaceae	100	0
Crotalaria mucranata Desv.	Fabaceae	96	4
Datura stramonium L.	Solanaceae	.0	100

Contd...

Table 4.2.2 contd.: Effect of different plant extracts on spore germination of *Alternaria alternata*

Name of plants	Family	*Alternaria alternata* (50 % alcoholic extract)	
		% Germination	% Inhibition
Datura metel L.	Solanaceae	0	100
Datura innoxia Mill.	Solanaceae	0	100
Elephantopus scaber L.	Asteraceae	64	36
Phyllanthus emblica L.	Euphorbiaceae	86	14
Eucalyptus globosus Labill.	Myrtaceae	20	80
Euphorbia hirta L.	Euphorbiaceae	96	4
Heliotropium indicum L.	Boraginaceae	100	0
Hibiscus rosa-sinensis L.	Malvaceae	97	3
Holarrhena antidysentrica Wall.	Apocynanceae	27	73
Hyptis suaveolens (L.) Poit.	Lamiaceae	22	78
Lantana camara L.	Verbenaceae	78	22
Lagerstroemia speciosa L. Pers.	Lythraceae	77	23
Mangifera indica L.	Anacardiaceae	88	12
Melastoma malabathricum L.	Melastomaceae	52	48
Melia dubia Cav.	Meliaceae	13	87
Mimosa pudica L.	Mimosaceae	78	22
Mitracarpus verticillatus (Schumach&Thonn) Vatke	Rubiaceae	77	23
Moringa oleifera Lamk.	Moringaceae	35	65
Murraya koenigi (L.) Spreng	Rutaceae	26	74
Nyctanthes arbor-tristis L.	Nyctanthaceae	10	90
Ocimum gratissimum L.	Lamiaceae	12	88
Ocimum sanctum L.	Lamiaceae	28	72
Oldenlandia corymbosa L.	Rubiaceae	85	15
Oxalis corniculata L.	Oxalidaceae	100	0
Phyllanthus fraternus Webster.	Euphorbiaceae	100	0
Piper betel L.	piperaceae	23	77

Contd...

Table 4.2.2 contd.: Effect of different plant extracts on spore germination of *Alternaria alternata*

Name of plants	Family	*Alternaria alternata* (50 % alcoholic extract)	
		% Germination	% Inhibition
Plumeria rubra L.	Apocynanceae	10	90
Polyalthia longifolia Sonnerat	Annonaceae	15	85
Pouzolzia indica L.	Utricaceae	100	0
Psidium guajava L.	Myrtaceae	20	80
Raphanus sativus L.	Brassicaceae	100	0
Rauvolfia tetraphylla L.	Apocynanceae	80	20
Ricinus communis L.	Euphorbiaceae	98	2
Saraca asoca (Roxb.) De Wilde	Caesalpiniaceae	100	0
Scoporia dulcis L.	Scrophulariaceae	24	76
Sida acuta L.	Malvaceae	72	28
Smilax zeylanica L.	Smilaceae	95	5
Solanum khassianum Clark	Solanaceae	80	20
Solanum nigrum L.	Solanaceae	100	0
Solanum torvum Sw.	Solanaceae	85	15
Solanum xanthocarpum Schrad & Wendl.	Solanaceae	90	10
Tridax procumbens L.	Asteraceae	80	20
Syzygium cumini (L.) Skeels	Myrtaceae	80	20
Tectona grandis L.f.	Verbenaceae	78	22
Terminalia arjuna (Roxb). W&A	Combretaceae	92	8
Vitex negundo L.	Verbenaceae	30	70
Xanthium strumarium L.	Asteraceae	0	100
Zingiber officinale Roscoe	Zingiberaceae	18	72

Data are average of 300 spores and rounded to nearest whole number

4.2.3: Effect of antifungal activity of selected plant extracts on the growth of the *Alternaria alternata* (following Poison food technique).

On the basis of spore germination bioassay five plants (*X. strumarium*, *Clerodendrum viscosum*, *Allium sativum*, *Polyalthia longifolia*, *D. stramanium*) were selected for further experiments. Antifungal activity of the selected plant extracts (aqueous, ethanolic and ethylacetate extracts) were tested for mycelia growth inhibition of *Alternaria alternata*. In these experiments plant extracts were mixed with potato dextrose agar media and after

solidification mycelial blocks were placed on the plant extract supplemented media. Details of the experiments have been discussed in the section 3.11 of materials and methods. Results of the radial growth of the fungi were noted and have been presented in Table 4.2.3.

From the results it was evident that leaves extracted in distilled water, ethanol and ethylacetate exhibited significant inhibitory effect against *A. alternata*. Aqueous and ethanolic leaf extracts of *X. strumarium*, *Clerodendrum viscosum*, *Allium sativum*, *Polyalthia longifolia* and *D. stramonium* showed less activity than the leaf extracts made in ethyl acetate. In antifungal assay agar *A. sativum* leaf extract (ethyl acetate extract) showed 100% inhibition of radial mycelial growth against *A. alternata*. Ethyl acetate extracts of *X. strumarium*, *C. viscosum*, *P. longifolia* and *D. stramanium* also showed antifungal growth inhibitory activity of *A. alternata* (Inhibition was 57.7%, 75.5%, 73.3%, and 62.2 % respectively).

Table 4.2.3 : Effect of antifungal activity of selected plant extracts on the growth of *A. alternata*, a pathogen of tomato plants(following poisoned food technique).

Plants Used	Leaves extracted in the Solvent	*Alternaria alternata*	
		Radial growth (mm)*	Percent inhibition
Xanthium strumarium	Aqueous extract	27	40.0
	Ethanolic extract	24	46.6
	Ethyl acetate	19	57.7
Clerodendrum viscosum	Aqueous extract	22	51.1
	Ethanolic extract	18	60.0
	Ethyl acetate	11	75.5
Allium sativum	Aqueous extract	12	73.3
	Ethanolic extract	08	82.2
	Ethyl acetate	00	100.0
Polyalthia longifolia.	Aqueous extract	39	13.3
	Ethanolic extract	23	48.8
	Ethyl acetate	12	73.3
Datura stramonium	Aqueous extract	32	28.8
	Ethanolic extract	18	60.0
	Ethyl acetate	17	62.2
Control	-	45	-

Control diameter = 45 mm; Data are mean of three replications; PDA : extract = 9:1; *Data were taken after 5 days of incubation. Percent inhibition were calculated in relation to control (where no inhibition of growth was recorded).

4.2.4: Agar cup bioassay for detection of antifungal activity by different plant extracts.

After spore germination bioassay, some plant extracts were made in soxhlet using benzene as solvent. Extraction procedure in soxhlet has been described in the materials and methods section 3.8.5. These plant leaf extracts were subjected to agar cup bioassay against *A. alternata.* The minimum inhibitory concentration of the extracts was also determined against the pathogen of tomato. The details of the procedures of agar cup bioassay have been presented in the materials and methods section section 3.13. Diameter of inhibition zones of different extracts at different concentrations were noted in the table 4.2.4. Different concentrations of the extracts were prepared and poured in agar cups or wells (5 mm. in diameter) and the lowest concentrations which showed inhibition were considered as MIC of the respective extracts.

Table 4.2.4: Bioassay of leaf extract (Benzene soxhlet extract) *Datura stramonium* against mycelia growth of *Alternaria alternata* (following agar cup method)

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Benzene soxhlet extract of: *Datura stramonium*	0.2	13
	0.1	12
	0.08	10
	0.06	8
	0.04	7
	0.02	6
	control	00

Experiment was performed in PDA medium

Results presented in table-4.2.4 clearly indicated the antifungal activity of *D. stramonium* leaf extract against *A. alternata.* Six different concentrations (0.2, 0.1, 0.08, 0.06, 0.04, and 0.02 gm/ml) of the leaf extract *D. stramonium* were prepared by serial dilution of the stock. All the concentrations were tested for their MIC values. The MIC value of *D. stramonium* leaf extract was 0.02gm/ml against *A.alternata.* Like the *D. stramonium,* leaves of *B. alata* were also extracted in Chloroform and were tested for their antifungal efficacy against mycelia growth of *A. alternata.* From the results (table 4.2.5) the minimum inhibitory concentration was found to be 0.006 gm/ml against mycelial growth of the *A. alternata in vitro.* After *B. alata,* leaves of *Annona squamosa* in 4 different concentrations (0.1, 08, 0.06 and, 0.04gm/ml) were tested against *A. alternata.* The MIC values of the

Annona squamosa leaf extract *was 0.06gm/ml* against *A. alternata.* Five different concentrations (0.1, 0.06, 0.01, 0.008, and 0.005gm/ml) of the *Ocimum gratissimum* leaf extract were also tested to determine MIC value. The MIC value of the *O. gratissimum* leaf extract was 0.008gm/ml against *A. alternata.* Results presented in table-4.2.8 clearly indicated the antifungal activity of *X. strumarium* leaf extract against *A. alternata.* Five different concentrations (0.3, 0.2, 0.1, 0.08 and 0.06 gm/ml) of the *X. strumarium* leaf extract were tested. The MIC value of *X. strumarium* leaf extract was 0.06 gm/ml against *A. alternata.*

Table 4.2.5: Determination of MIC of leaf extract of *Borreria alata* (Chloroform extract made by soxhlet) against mycelial growth of *A. alternata* (following agar cup method)

Plant extract using soxhlet	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Chloroform extract of: *Borreria alata*	o.1	15
	0.06	14
	0.01	12
	0.008	11
	0.006	9
	control	00

Experiment was performed in PDA medium

Table 4.2.6: Bioassay of leaf extract (Chloroform soxhlet extract*) Annona squamosa* against mycelia growth of *Alternaria alternata* (following agar cup method)

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Chloroform soxhlet extract of: *Annona squamosa*	0.1	10
	0.08	8
	0.06	6
	0.04	0
	control	00

Experiment was performed in PDA medium

Table 4.2.7: Bioassay of leaf extract (Chloroform extract by soxhlet method) *Ocimum gratissimum* against mycelia growth of *A. alternata* (following agar cup method)

Plant extract	Extract Concentration (gm/ml)	Inhibition zone diameter (mm)
Chloroform soxhlet extract of: *Ocimum gratissimum*	0.1	10
	0.06	8
	0.01	7
	0.008	6
	0.005	0
	control	00

Experiment was performed in PDA medium

Table 4.2.8: Bioassay of leaf extract (Benzene soxhlet extract) *X. strumarium* against mycelia growth of *A. alternata* (following agar cup method)

Plant extract	Extract Concentration (g/ml)	Inhibition zone diameter (mm)
Benzene soxhlet extract of: *Xanthium strumarium*	0.3	16
	0.2	12
	0.1	10
	0.08	9
	0.06	8
	control	00

Experiment was performed in PDA medium

4.2.5: Extraction of terpenoid fraction of *Datura stramonium* bioassay of the fractions against *Alternaria alternata* (following disc diffusion bioassay).

D. stramonium was selected for separation of terpenoids from the mixture of compounds present in its leaf extract. Details of the isolation procedures have been discussed in the materials methods table-3.8.6. Details of the procedure of disc diffusion bioassay have been presented in section 3.12 of materials and methods. MIC values of terpenoid fractions of *D. stramonium* were determined against *A. alternata.*

Results presented in table-4.2.9 clearly indicated the antifungal activity of *D. stramonium* leaf extract against *A. alternata.* Five different concentrations (3.0, 2.0, 1.0, 0.50, 0.25 mg/ml) of the leaf extract of *D. stramonium* were prepared by serial dilution of the stock. The MIC value of *D. stramonium* leaf extract was 0.50mg/ml against *A. alternata.*

Table 4.2.9: Determination of MIC of terpenoid fraction of leaf extract of *D. stramonium* against growth of *A. alternata* (following disc diffusion method).

Plant leaf extract	Discs dipped in extract Concentration (mg/ml)	Inhibition zone diameter After 48 hours (mm)
Ethyl acetate extract. of *Datura stramonium*	3.00	12
	2.00	11
	1.00	08
	0.50	07
	0.25	00
	control	00

Experiment was performed in PDA medium

4.2.6: Extraction of antifungal properties in three different solvents and antifungal assay:

Selection of the solvent is one of the important aspects for soxhlet extraction. In the present section three different solvents were used to know the best solvent to be used in future for extraction of the active antifungal principles. Five plants were selected separately for pathogens of tomato and the extracted compounds were subjected to antifungal bioassay at same concentration. On the basis of the results the suitable solvents were selected for future extraction purpose. From the table 4.2.10 it is clear that benzene was the best solvent for extraction of the antifungal properties among the five plants tested. Details of the results have been shown in table 4.2.10. *X. strumarium, B. alata, Annona squamosa* and *D. stramonium* extracted in benzene and chloroform showed almost same result but benzene was found better for extraction of *Clerodendrum viscosum* leaves.

Table 4.2.10: Antifungal activity of five plant extracts (prepared in soxhlet using three different solvents) against *Alternaria alternata* (following agar cup method)

Plant Extract*	Different solvent	Diameter of Zone
Xanthium strumarium	Benzene	17
	Chloroform	17
	Hexane	12
Borreria alata	Benzene	15
	Chloroform	15
	Hexane	10
Annonus squamosa	Benzene	13
	Chloroform	13
	Hexane	06
Clerodendrum viscosum	Benzene	09
	Chloroform	07
	Hexane	00
Datura stramonium	Benzene	14
	Chloroform	14
	Hexane	06
Control	Benzene	0
	Chloroform	0
	Hexane	0
	Water	0

* concentration used 0.3g/ml

4.2.7: Antifungal sensitivity assay of three common fungicides.

Three commonly used fungicides were tested for their efficacy against the pathogen of tomato. Bioassay was done taking three to four different concentrations of three different fungicides nysyatin, captaf and bavistin. The three different concentrations for captaf and four different concentrations for nystatin and bavistin were selected on the basis of their activity for determination of minimum inhibitory concentrations of the fungicides against the fungus. Minimum inhibitory concentrations (MIC) were measured following standard techniques (Suleman *et al.* 2002) as described in the materials and methods (section 3.13). The fungal pathogens showed growth inhibition zones around agar cups containing effective concentrations of the fungicides. The lowest concentration which could inhibit growth of a fungi, were considered as MIC of that fungicide against the fungi tested.

Different concentrations of the fungicides were prepared by serial dilution of the fungicide solutions. Separate PDA plates, after solidification, were inoculated with 1 ml of spore suspension (*A. alternata*). Spore suspension was spreaded uniformly on the agar surface by a glass spreader. Three to four agar cups or wells (of 5 mm diameter) were made on the inoculated plates aseptically and 50 µl fungicides of different concentrations were poured in the wells. The wells were marked and the plates were incubated at 28 $\pm 1^0$ C in an incubator. The plates were observed after two days of inoculation and the least concentration in which the pathogenic fungi could not grow were considered as minimum inhibitory concentration. The results of agar cup bioassay against fungal the pathogen has been presented in table 4.2.11.

Four different concentrations of fungicide (nystatin) were assessed for their efficacy against *A. alternata*. Minimum inhibitory concentration of *A. alternata* was found to be 3 mg/ml (table 4.2.11). Similarly, fungicides captaf was also used to control the pathogen and the MIC value of captaf were 5 mg/ml against *A. alternata*. Bavistin was also used to control the pathogen. Bioassay studies of bavistin against *A. alternata* showed minimum inhibitory concentration of 3 mg/ml. Antifungal sensitivity of different concentrations of the fungicides have been shown in the plate-30.

Table 4.2.11: Inhibition of growth of *A. alternata* by different concentrations of Nystatin, Captaf and Bavistin (following agar cup method).

Fungicide	Concentration (mg /ml)	Diameter of inhibition zone (mm)
Nystatin	5	10
	3	8
	1	0
	0.1	0
Captaf	10	18
	5	12
	2	0
Bavistin	5	8
	3	6
	1	0
	Control	0

Experiment was performed in PDA medium

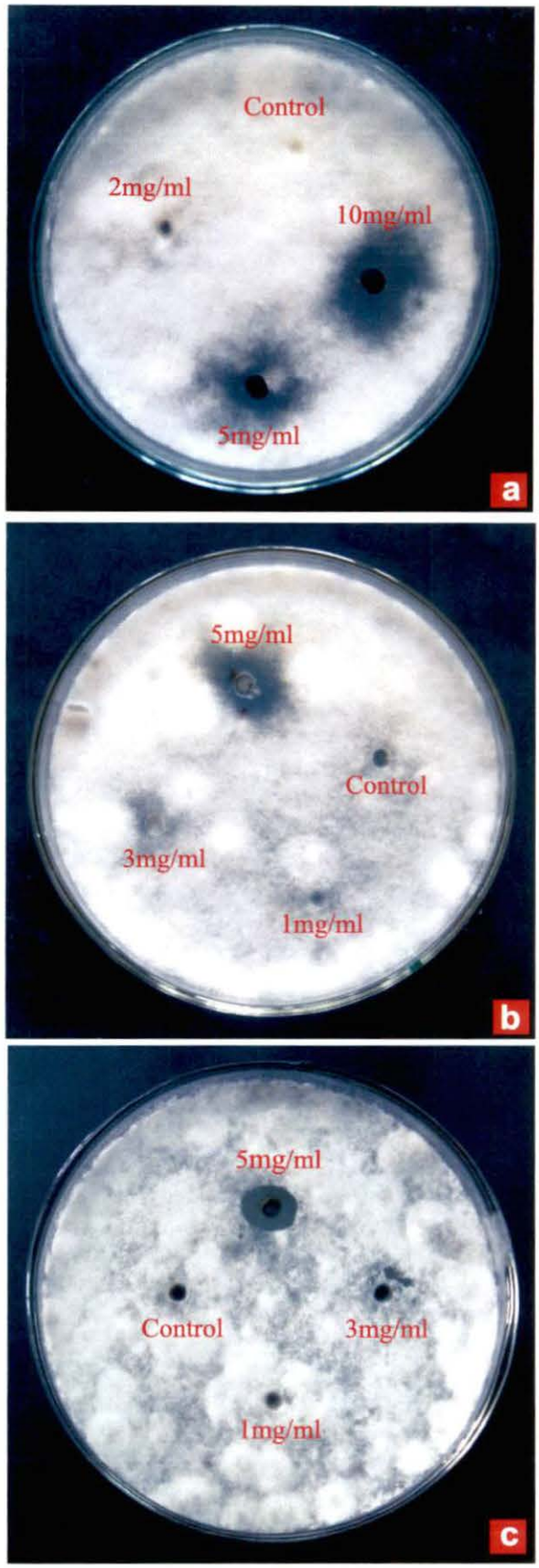

Plate30: Fungicidal activity against *Alternaria alternata*
fig. a. Captaf used as fungicide at different concentrations
fig. b. Bavistin used as fungicide at different concentrations
fig. c. Nystatin used as fungicide at different concentrations.

4.2.8 : Separation of plant extracts on TLC plates developed in suitable solvent and bioassay of the plates for antifungal properties against *Alternaria alternata.*

Like the previous chapter (section 4.1.11) two plants (*X. strumarium* and *C. viscosum*) were selected for TLC plate bioassay against *A. alternata* and for "on the chromatogram" analysis of the antifungal compounds. The selected extracts were run on previously activated TLC plates and the plates were developed separately in two solvents [either (Hexane:Ethylacetate:Methanol::60:40:1) or (Chloroform : Methanol :: 9:1)]. The plates were dried in the air at room temperature (28^0 C). Spores of *A. alternata* were mixed with Richard's solution and finally the mixed suspension was sprayed on the silica gel coated plates. The plates were placed in a humid chamber at 25 ± 1^0C for 2-3 days. Details of the techniques have been presented under materials methods (section-3.15.1.). After 3 days the inhibition zones were observed (plate 31 & 32) and the diameter of the inhibition zones was measured. The R_f of the inhibition zones were calculated and have been presented in the tables 4.2.12. The *X. strumarium* plant extract tested against the pathogen have shown one distinct antifungal zone (plate-31) at R_f 0.85. The diameter of the antifungal zone was 20mm. The plant extract of *C.viscosum* tested against the pathogen have shown two antifungal zones (plate-32) at R_f 0.68 and 0.98. The diameter of the antifungal zones was 33 and 6 mm respectively.

The bioassay guided fractions were subjected to chromogenic spray with four different reagents separately. The details of the chromogenic analysis have been presented in table-4.1.13. Details of the reagent preparation and application along with the table of analysis (Table-3.4) have been presented in the materials and methods section-3.16.

The antifungal zone shown by *X. strumarium* against the pathogen seems to be bitter principles because dark-green colour was observed after anisaldehyde reagent spray (Plate 24 of chapter I). The same zone in separate plate when sprayed with Vanillin-sulphuric acid mixture became light green which confirmed bitter principle.

Both antifungal zones shown by leaf extract of *C. viscosum* were dark green in colour for bitter principle. Two other spray reagents (10% KOH and Dragendorff reagent) were also sprayed but they could not produce any significant colours for identification of the compounds.

Plate 31: Bioassay of crude leaf extract of *Xanthium strumarium* developed in chloroform: methanol (9:1) and sprayed with Richard's solution supplemented with spore suspension of *Alternaria alternata*. Antifungal zone in parentheses along with R_f value has been presented.

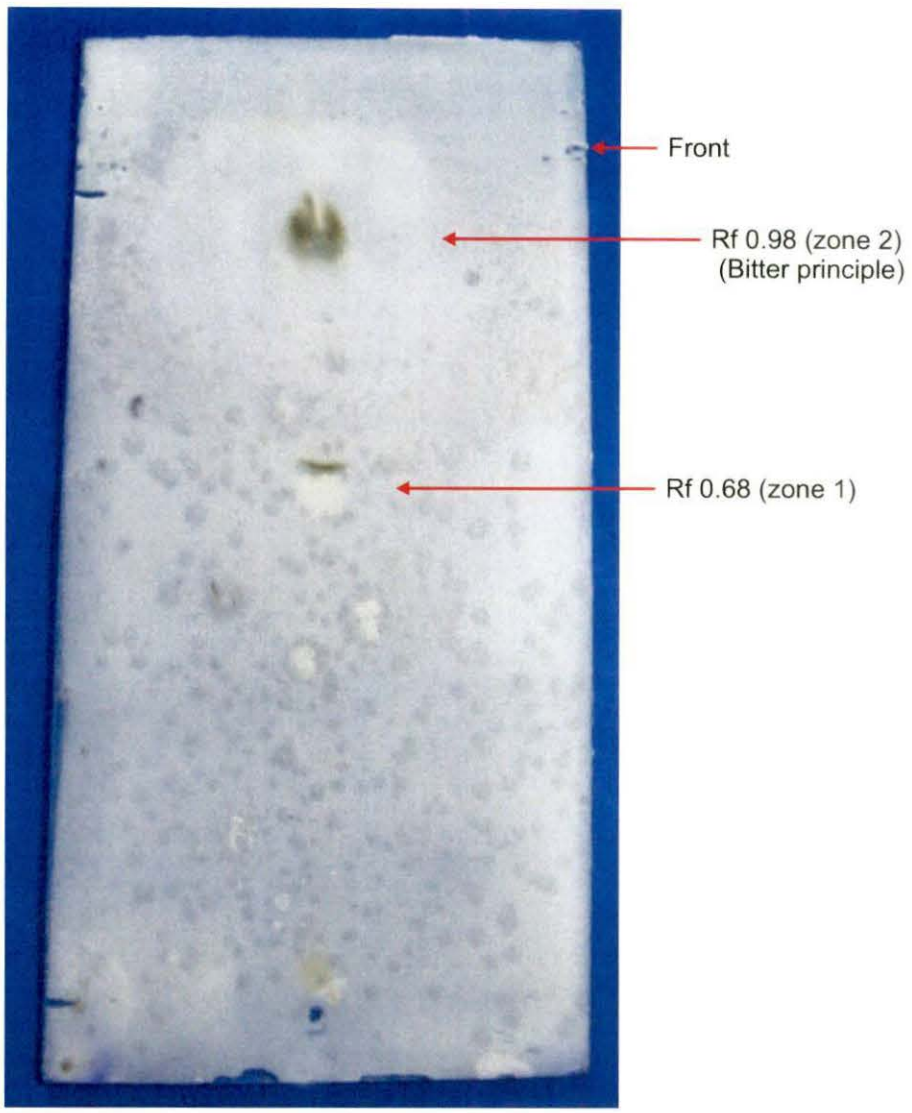

Plate 32: TLC plate bioassay of crude leaf extract (soxlet extract in benzene) of *Clerodendrum viscosum*. The plate was developed in chloroform: methanol (9:1) and sprayed with Richard's solution supplemented with spore suspension of *A.alternata*. Two antifungal zones were observed (zone 1&2) against *A.alternata*. R_f of the zones have been presented following chromogenic spray. The zones were identified as bitter principles.

Table 4.2.12 : TLC plate bioassay of crude leaf extracts of two different plants against *Alternaria alternata*

Extracts of the plants	No. of inhibition Zones	*Alternaria alternata*	
		Diameter of Inhibition zones (mm)	R_f
Xanthium strumarium	Zone 1	20	0.85
Clerodendrum viscosum	Zone 1	06	0.98
	Zone 2	33	0.68

Table 4.2.13: Chromogenic analysis of crude leaf extracts of two different plants against *Alternaria alternata.*

Extracts of the plants	TLC plate bioassay guided antifungal zones and their R_f in parentheses	*Spray reagent*			
		Anisaldehyde		*Vanillin H_2SO_4*	
		Colour	Probable compound	Colour	Probable compound
Xanthium strumarium	Zone-1(0.85)	Dark green	Bitter principle	Blue-green	Bitter principle
Clerodendrum viscosum	Zone-1(0.98)	Dark green	Bitter principle	NP	-
	Zone-2(0.68)	Dark green	Bitter principle	NP	-

*Crude leaf extract =100 µg/ml; ND = Not defined; NP=Not performed;TLC plates developed in solvent = Hexane:Ethylacetate:Methanol :: 60:40:1

4.2.9: *In vivo* control of foliar pathogens of tamato by application of plant extracts.

Antagonistic activity of aqueous extracts (0.5g / ml distilled water) of *D. stramonium* and *X. strumarium* were tested *in vivo* on a susceptible tamato variety (Karan). The plants were sprayed with crude aqueous extracts in separate sets. In control set, sterile distilled water was sprayed. After 24 hours, the plants in each set were inoculated with *A. alternata* spore suspension. The details of the experimental techniques etc. have been presented in materials and methods (section-3.6.3 and 3.7.2). Results of the experiments have been presented in the table-4.2.14.

From the results (table-4.2.14) it was evident that 2 plant extracts tested showed significant reduction in mean disease index. When *D. stramonium* was pre-sprayed it could control the disease completely, in comparison to control plants. Next to *D. stramonium*, *X. strumarium* also reduced *Alternaria* blight disease significantly (up to 83% after 10 days of

inoculation). After 10 days of incubation in control plants disease index was 7.1. The disease index was reduced to 1.2 when plants were sprayed with *X. strumarium.*

Table 4.2.14: *In vivo* control of blight of tomato (cv Karan) caused by *Alternaria alternata* by foliar application of plant extracts of *Datura stramonium and Xanthium strumarium*

Extracts of the plants	Mean disease index/plant*				
	Incubation period (Days)				
	2	4	6	8	10
D. stramonium	0	0	0	0	0
X. strumarium	0	0	0	0	1.2±0.15
Control	0	1.8±0.37	3.0±1.24	5.1±1.92	7.1±2.37
CD at 5%	0	-	-	-	3.1

*Mean of three replications. Data after ± represent standard error values. - = not performed.