MANAGEMENT OF EUPATORIUM [*Chromolaena odorata* (L.) King and Robinson] WEED THROUGH MYCOHERBICIDES

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SEPTEMBER, 2001

MANAGEMENT OF EUPATORIUM [*Chromolaena odorata* (L.) King and Robinson] WEED THROUGH MYCOHERBICIDES

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in partial fulfilment of the requirements for the
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in

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By

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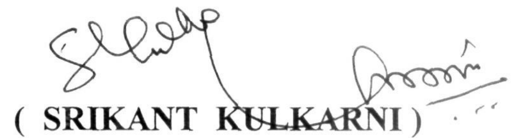

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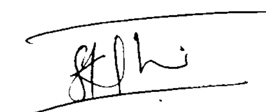

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INTRODUCTION

I. INTRODUCTION

Chromolaena odorata (L.) King and Robinson (= *Eupatorium odoratum* L.) commonly known as Eupatorium sp. is an abnoxious weed, belonging to the order-Asterales, Family- Asteraceae, Tribe - Eupatorieae, genus - Eupatorium (Tourn) L. Among 1200 species, only eight are being present in India viz., *Eupatorium adenophorum L., E. capillifolium L., E. chinense L., E. lingutrium L., E. nodiflorum L., E. odoratum L., E. riparium L.,* and *E. triplinerve L.,* (Malhotra and Jain, 1978). *C. odorata* (Siam weed) and *C. adenophorum* (Crofen weed) are the noxious weeds in many tropical countries of the world (Rai, 1976).

It is an alien weed, a native of West Indies and continental America. It entered India as an ornamental plant in to the Royal Botanical Gardens of Kolkata during 1881(Biswas, 1934). Later, it migrated to Assam (1914), West Bengal (1925) and Kerala (1942). Further, it spread rapidly to all the southern states of India.

In India, the weed is now very well distributed in north-eastern and southern states particularly in Assam, West Bengal, Orissa, Karnataka, Goa, Maharashtra, TamilNadu, Kerala and all along the western ghats.

Chromolaena odorata is highly aggressive, invasive and adoptive to wide agro-ecological conditions. It is a perennial herbaceous shrub growing up to eight metre with a massive tap root system, there by drawing the nutrients and competing with the surrounding vegetation. It has high reproductive capacity and can also propagate vegetatively. It smothers other vegetation wherever, it grows and is capable of suppressing even some of the aggressive cover crops, as it possesses allelopathic potentialities and growth inhibitors (Ambica and

Jayachandra, 1980). These attributes contributed to its successful spread and establishment as a 'weed'.

The weed *C. odorata* has occupied pastures, marginal lands, open areas, dry deciduous forest and interior shrub jungles, where it is highly competitive and does not let other flora and grass to grow. It has also become a menace in coconut, arecanut, tea, teak, coffee, cardamom, rubber, oil palm and other plantations.

In forest ecosystems, it decreases the value of timber, forest seed, orchards and increases the cost of seedling production in nurseries there by decreasing the over all productivity of forest ecosystem. Dry stalks of the weed cause fire hazards in forests during summer season. The weed pose a grave threat to fragile bio-diversity of the Western ghats, where it is competitively replacing the existing indigenous rich flora, there by creating ecological imbalance. It also suppresses the plantation crops at their early stages due to its allelopathic effects (Ambica and Jayachandra, 1980). The forest department of Karnataka spends several lakhs of rupees annually to clear this weed in the nurseries and young plantations, but the problem has remained unchecked and also severe.

All these points highlight *C. odorata* as a threat to agriculture and environment, there by stresses an urgent need to take steps to manage the weed, so as to maintain the ecological integrity.

Widely adopted conventional methods like cutting and burning has got many limitations and uneconomical due to its perennial nature and resprouting capacity. Chemical control, through use of herbicides is not only a costly affair but also causes environmental pollution, human and animal health hazards.

Hence, the only way left out is to find economically sound and environmentally safe practical method of control i.e 'Biological control'.

However, biocontrol attempts to manage *C. odorata,* through insect *Pareuchactes pseudoinsulata* became futile due to its failure to establish under field conditions (Subbaiah, 1992).

Keeping these points in view, the present investigation was undertaken to exploit the fungal pathogens associated with *C. odorata* and to manage the weed through mycoherbicidal approach with the following objectives.

1. Survey and surveillance in the endemic areas of *Chromolaena odorata* (*Eupatorium)* weed and also collection of isolates of different pathogens.

2. Screening of different pathogens for their efficacy in inducing disease in Eupatorium weed and study of morphological, cultural, physiological, nutritional and biochemical characteristics of the pathogen.

3. Standardization of inoculation techniques and their evaluation in *in vitro* and *in vivo.,* and

4. Mass production of pathogens and their field efficacy in managing the weed, *Chromolaena odorata.*

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Weeds are always menace to mankind. Many weed species remain refractory to conventional strategy of weed control. Biological weed control is an approach utilizing living organisms to control or reduce the population of weeds.

One of the methods of biological control of weeds is the 'mycoherbicidal strategy'. Daniel et al (1973) introduced the concept of mycoherbicide and demonstrated that, an endemic pathogen might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particular vulnerable stage of weed growth.

Templeton *et al.* (1979) have coined the term 'mychoerbicides' for fungi used as biological herbicides. Mycoherbicide is defined as 'Plant Pathogenic fungi developed and used in the inundative strategy to control weeds in the way chemical herbicides are used'. (TeBeest and Templeton, 1985).

Although *Chromolaena odorata* has been the subject of biocontrol programme for over 20 years (Cruttwell, 1974; Cock, 1984), the emphasis has been made on the use of arthropod agents (Julien, 1992) and least attention has been given to fungal pathogens (Ooi *et al.,* 1991). This indicates the cradle stage of mycoherbicidal control approach. The literature available is confined only to survey and morphological studies of a few pathogens associated with *C. odorata.* Hence, in this chapter mycoherbicidal control of other weeds has been reviewed wherever the literature on *C. odorata* is not available.

2.1 Survey and surveillance on pathogens of *Chromolaena odorata (Eupatorium)*

Evans (1987) carried out both literature and herbarium survey of fungal pathogens recorded on *Chromolaena odorata*, while Chacko and Narasimhan (1988) highlighted the need for systematic survey of pathogens in the centre of origin. Russo (1985) reported the possibility of bio control of *C. odorata* in Gaum, using a leaf spot causing fungus *Septoria* sp. which produced necrotic leaf spots with red margins.

Elango *et al.* (1993) carried out a pathological survey of *C. odorata* in Trinidad and Tobago, and reported few leaf spots inducing fungi viz., *Septoria ekmaniana, Anthelia niger, Mycovellosiella perfoliata* and one rust pathogen *Cinothrix praelonga.* Barreto and Evans (1994) undertook a two year survey (from Oct 1988 to Aug. 1989) of fungi associated with *C. odorata* in Southern Brazil. Samples of all fungi suspected of being pathogenic to *C. odorata* were collected and isolations were made on V-8 juice agar or potato dextrose agar. Different fungal pathogens isolated and the typical symptoms produced on the weed are tabulated below. Amongst, A*nhellia niger, Mycovellosiella perfoliata* and *Septoria ekmaniana* were considered to be promising classical biocontrol agents of *C. odorata.*

The mycobiota of the weed *Chromolaena odorata* in the Southern Brazil as given by Barreto and Evans (1994).

Sl.No.	Fungal Pathogen	Symptoms
1.	*Alternaria zinniae* M.B. Ellis	Leaf lesions 2-20 mm wide, dark brown, angular when vein delimiting along leaf margins or forming round to elongate brown spots with black periphery on central leaf lamina.

2.	*Anhellia niger* (Viegas) Arx.	Lesions on stems, leaves, petioles and flower peduncles: Brown, angular, extensive, destroying large areas on whole leaves on affected stems, fructifications formed resulting in scaby growth.
3.	*Mycovellosiella perfoliata* (Ellis & Everh.)Munt-Cvetk.	Lesions on leaves, 2-25 mm wide, irregular in shape, initially indistinct becoming well defined with raised margins, greyish brown adaxially, brown adaxially, necrotic area rupturing and falling giving shot hole symptoms.
4.	*Ophiociliomyces bauhiniae* A.C. Batista & H. Lima	Lesions absent, fungus form sooty cover on aerial parts.
5.	*Pseudocercospora eupatorii - formosanii* (Sawada) J.M. Yen	Lesions on leaves 2-10 mm diameter, greyish brown, circular to angular, vein-delimiting with raised margins; coalescing and forming extensive necrotic zones which lead to leaf death.
6.	*Redbia trichomambusta* R.W. Barreto sp. nov.	Conidiophore and conidia grouped on leaf hairs imparting burnt appearance.
7.	*Septoria ekmaniana* Petr. & Cif.,	Leaf lesions 1-2 mm diam, initially punctiform becoming roundish, forming isolated to abundant spots, later coalescing to cover whole leaves: Greyish brown to black periphery.
8.	*Fusarium pallidoroseum* (cooke) Sacardo	Black stromatic structures erupting through the epidermis of stems and branches. Pathogenic status has not been proven. Its true role as a bio control agent of *C. odorata* is doubtful.
9.	*Cinothrix* Praelonga (winter) Arthur	Yellowish orange rust pustules on leaf lamina.

Fungal Pathogens recorded on *C. odorata* (Based on CABI Database, Herb. IMI)

Sl. No.	Fungal Pathogens	Scientist/ Source	Distribution
A. Ascomycetes & Deuteromycetes			
1.	*Acrostalaymus albus* Preuss	Viegas (1961)	South America
2.	*Anhellia niger* (Viegas) Arx	Stevens (1930)	Guyana
3.	*Appendiculella sororcula* (Speg) H.	Ciferri (1961)	Dominican Rep.
		Leather (1967)	Jamaica
		Urtiaga (1986)	Venezuela
		Baker & Dale (1951)	Trinidad & Tobago
4.	*Byssophaeria schiermayeriana F.*	IMI 157432	Brazil
5.	*Capnodium* sp.	Urtiaga (1986)	Venezuela
6.	*Cercospora* sp.	Johnston (1960)	Malaysia
		Puckdeedindan (1966) IMI 194503	Thailand
			Nepal
7.	*Cercospora aciculina* chupp.	IMI 176830 Litzenberger *et al.*, (1962)	Nigeria Cambodia
8.	*Cercospora eupatorii* peck	Litzenberger *et al.*, (1962)	Cambodia
		Urtiaga (1986) Evans (1987)	Cuba
			Nepal, Hawaii
			N.America, Ivory cost
9.	*C. eupatorii odorati* yen.	Yen 1968)	Malaysia
10.	*Chaetothyrium dominicanum ciferri*	Ciferri (1961)	Dominican Rep.

11.	*Colletotrichum sp.*	Litzenberger *et al.*, (1963)	Cambodia
12.	*Cylindrosporium* sp.	Litzenberger *et al.*, (1963)	Cambodia
13,	*Glomerella cingulata* (Stonem)S & H. Schrenk	Urtiaga (1986) IMI145666	Cuba Srilanka
14.	*Guignardia eupatorii* punith	Punithalingam (1974)	Srilanka
15.	*Hormodendron (cladosporium) eupatorii* cif	Ciferri (1961)	Daminican Rep.
16.	*Mycosphaerella eupatorii* Yen.	Yen (1979)	Malaysia
17.	*Mycosphaerella fungurahuana* Sydow.	Urtiaga (1986)	Venezuela
18.	*Ophiobolus ipohensis* Yen.	Yen (1979)	Malaysia
19.	*Ophiociliomyces bauhiniae Yen.*	Litzenberger *et al.*, (1962)	Cambodia
20.	*Ophiosphaerella eupatorri Yen.*	Yen (1979)	Malaysia
21.	*Phaeosphaeria eupatoriicola Yen.*	Yen (1979)	Malaysia
22.	*Phomopsis eupatoriicola* Petrak	IMI 145669	Srilanka
23.	*Phloeospora* sp.	Urtiaga (1986)	Venezuela
24.	*Phyllosticta eupatorii cola* Yen	Yen (1979) Viegas (1961)	Malaysia South America
25.	*Pseudocercospora eupatorii formosani* (Sawada) Yen	Peregrine and Ahmad (1982) Evans (1987) Yen (1968)	Borneo Brunei Burma, Thailand Malaysia
26.	*Septoria* sp.	IMI 262423	Bangaladesh
27.	*Septoria ekmaniana*	Russo (1985) Ciferri (1961)	Gaum Dominicap Rep.

28.	*S. eupatorii* R & D	Urtiaga (1986)	Venezuela
29.	*Setella citricola sydow*	Ciferri (1961)	Dominican Rep.
B. Basidiomycetes			
30.	*Cinothrix praelonga*	Evans (1987) Baker and Dale (1951) Gallego & Cummins (1981)	Dominica Venezuela Trinidad & Tobago Mexico
31.	*Coleosporium eupatorii Arthur*	Tai (1979)	China
32.	*Coleosporium steviae* Arthur	Gallego & cummins (1981)	Mexico
33.	*Thanatephorus cucumeris* (Frank) Donk	Sawada (1931)	Taiwan
34.	*Uredo bullula* Kern	Ciferri (1961)	Dominican Rep.

Fungal Pathogens reported from India on *C. odorata*

Cercospora eupatorii peck	Subramanian and Tyagi (1964)
Didymosphaeria sp.	India IMI 312419
Meliola sp.	India IMI 237720
Pseudocercospora eupatorii Formosanii (Sawada) Yen	Evans (1987)

Fungal Pathogens reported from India on eupatorium sp. (Pers. Comm.)

Cercospora assamensis Chowdhary.

C. eupatoricola Govindu & Thirum.

Fusarium oxysporum Schl. Ex Fr.

Periconia cookei Mason & Ell.

Phoma eupatorii Died.

Similarly, many scientists reported several fungal pathogens on Eupatorium sp. The potentiality of *Cercospora eupatorii* as a bioherbicides on *Eupatorium adenophora* was reported from Queensland (Dodd, 1961).

Trujillo *et al.*,(1988) reported *Cercosporella ageratinae* sp. Nov. on *Eupatorium riparium.* Regel. Later this was described as *Entyloma ageratinae* (Barreto and Evans, 1988). *E. ageratinae* sp. nov. was released in Hawaii during 1975 by Hawaiian Dept.of Agric. U.S.A. in collaboration with University of Hawaii.The pathogen in combination with two insects *Procecidochares* utilis Stone. and *Oidaematophorus beneficus* provided substantial to complete control of *E. riparium.*

Morris (1991) reported the successful performance of *Entyloma ageratina* in South Africa after its release in 1989. It established well and caused extensive defoliation of *E. riparium.*

To combat *E. adenophorum* Spreng., *Phaeroramularia* sp. was intentionally released in South Africa (Morris, 1989). Hill (1989) reported the accidental introduction of *Cercospora eupatorii* peck to Australia from USA Via Hawaii and explained the possible role of the pathogen in biocontrol of *E. adenophorum.*

2.2 Morphological and cultural characteristics of pathogens

Barreto and Evans (1994) isolated and identified many fungal pathogens associated with *Chromolaena odorata* in Southern Brazil. They studied morphological and culture characters of isolated pathogens on V-8 juice agar/PDA medium to assess their biocontrol potentiality which is tabulated below.

Media preference by different mycoherbicides

	Pathogen	Host	Media tested	Best media	Autors
1.	*Ascochyta caulina* (P. Karst) v.d. Aa & V. Kest	*Chenopodium album* L.	Czapeckdox, malt meal agar, raw rye agar, fluid rye, clear v8 agar, wheat bran barley seeds	Oat meal agar Czapek's dox agar	Hortsten and Kempenaar (1994)
2.	*Colletotrichum* (Penz.) Sacc *gloeosporioides* f. sp. *aeschynomene*	Northern jointvetch (*Aeschynomena virginica* (L.) B.S. P.)	Various solid and liquid media	Limabean agar and V-8 juice	Daniel *et al.* (1973)
3.	*Fusarium pallidoroseum*	*Parthenium hysterophorus* L.	Richards's synthetic agar, PDA, Freshly prepared PDA, Hi-media brand of dehydrated PDA, Nutrient agar, corn meal agar, Kenknight Munaier medium, malt extract agar, potato carrot agar, oat meal agar, Czepekdox agar	Richards's synthetic agar and Malt extract agar	Anon. (1998)
4.	*Gliocladium virens*	*Parthenium hysterophorus* L.	Asthana and Hawkers media, Czepek's media, Richards media, Potato dextrose broth	Richards's media	Kauraw *et al.* (1988)
5.	*Nimbya scirpicola*	*Scirpus planiculamis* L.	Various solid and liquid media	Oat meal agar malt extract agar, Czapak Dox agar	Park Jong Han *et al.* (1995)

Cultural and morphological characteristics of pathogens of Chromolaena odorata

Sl. No.	Pathogen	Culture Characteristics	Colony diam (cms)
1.	*Alternaria zinniae*	Colony fast growing, Greyish to white, Cottony centre with dark grey to black felty margins. Produce conidia aerially.	4.2-4.7 cm after 7 days
2.	*Pseudocercospora eupatorii formosanii*	Slow growing colony, Felty aerial mycelium, greyish white surrounded by a wide flat pericentral zone with immersed mycelium. Poor/No sporulation	1.6-2.3 cm after 30 days
3.	*Anhellia niger*	Slow growing, Tar like with a raised centre and a flat area of sparse mycelium supporting tar like droplets of conidia. Mycelium black immersed	2.6 - 3.7 cm after 30 days
4.	*Rebdia trichomam busta*	Slow growing, felty centre of aerial mycelium, becoming sparse and predominantly submerged towards periphery. Dark grey with diurnal zonation. No sporulation but spermatial bodies occasionally produced.	2.5-3.7 cm after 30 days
5.	*Septoria ekmanian*	Colonies slow growing. Orange to pinkish centrally with light to chocolate brown periphery, Dark green to brown reverse. No sporulation but frequently produce spermatia	1.5-3.5 cm after 30 days

2.3 Physiological, nutritional and biochemical characteristics of pathogens

Hildebrand and Jensen (1991) studied the effect of temperature on growth of *Colletotrichum gloeosporioides* infecting st. John's Wort weed

(*Hypericum perforatum L.*) and found that optimum temperature for mycelial growth on agar was 24°C. On Weed host, *C. gloeosporioides* caused infection at 16°C, 22°C, 28°C after 25 h. 9h and 6h respectively.

Colletorichum capsici, a potential mycoherbicide of pitted morning glory, grew well at 20°C, 25°C and 30°C on PDA. Optimum temperature for conidial germination was 25 and 30°C (Cartwright, 1992).

Studies on the biological characteristics of *Mycovellosiella eupatorii - odorai* (Yen)Yen, revealed that, the pathogen required 25°C and pH 5.0 for conidial formation. And it was able to use all carbon sources and produced maximum growth in sucrose as carbon source and peptone as nitrogen source (Guo *et al.,* 1992).

Daigle and Cotty (1992) studied the factors influencing the germination and mycoherbicidal activity of *Alternaria cassiae* and observed that pH 6.5, 0.02 M Potassium phosphate buffer and 1 percent dehydrated PDB promoted the spore germination. Mortensen and Makowski (1994) reported better growth of *Colletotrichum trifolii,* a pathogen on red clover (*Trifolium pratense*) between 15 and 35°C.

Deshpande *et al.* (1997) tested a wide range of pH. 4.0 to 7.5 on *Alternaria alternata,* a pathogen of parthenium and reported luxuriant growth and sporulation.

2.4 Toxic metabolites

Many of the fungal pathogens of weeds are among the known genera that produce toxins, i.e., *Alternaria, Helminthosporium* (= *Exserohilum*) *Fusarium* etc. some of these toxins are host specific, others are not.

Fulton *et al.* (1965) and Templeton *et al.* (1967) observed that, chlorosis and mortality of early stage of seedlings was due to extra cellular metabolities of *Alternaria tenuis* Auct. Walker and Templeton (1978) suggested the concept of using phytotoxic metabolites for the biocontrol of weeds.

Alternaria crassa (Sacc.) Rands. on jinson weed (*Datura stramonium* L.) and *Alternaria helianthi* (Hansq.) Tubaki & Nishihara. on Cocklebur (*Xanthium strumurium* L.) produced a phytotoxic metabolite, which was characterised as 'radicinin'. (Quimby *et al.,* 1988). Further it was observed that, the crude extract of this metabolite delayed the seed germination of hemp susbania (*Sesbania exalta* L.)

Jones and Hancock (1990) reported the release of steroidal phytotoxin 'Viridol' from *Gliocladium virens* during pathogenesis against wide range of weeds. Strobel (1991) reported the production of a phytotoxin 'Maculosin' by *Alternaria alternata* (Fr.) Keissler for the control of *Centaurea maculosa* L.

Venkatasubhaiah and Chiton (1992) studied and characterised the different phytotoxins produced in culture filtrate of *Ascochyta hyalospora,* a leaf spot pathogen of *Chenopodium album* L. The different phytotoxins were *viz.,* 'ascochytin' 'pyrenolide A', and 'hyalopyrone'.

Septoria cirsii, a potential biocontrol agent of Canada thistle produced a phytotoxin, which was characterised as β-nitropropionic acid (Hershenhorn *et al.,* 1993).*Alternaria cassiae* produced two phytotoxins viz.,Ferricrocin and Coprogen against sickle pod (Ohra *et al.*, 1995)

Manickam *et al.* (1997) reported the inhibitory effect of *Fusarium moniliforme* culture filtrate on seed germination of *Parthenium hysterophorus* L. and suspected the presence of some toxin or toxin like substance in the filtrate. Saxena and Pandey (2000) reported the bioherbicidal properties of culture filtrate produced by two strains of *Alternaria alternata* LC-110 and LC-104 against *Lantana camera* L.

2.5 Host range studies

Host range testing schemes were developed for assessing the safety of non target host plants against pathogens (Wapshere, 1974, Charudattan, 1989).

Earlier research on the host range of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* revealed that, only the target weed *Aeschynomene virginica* (L.) BSP and a related weed *A. Indica* (L.) BSP were susceptible (Daniel *et al.,* 1973). Later, TeBeest (1988) tested 77 plant species and 43 genera in 10 families and found five genera in the sub family papilionideae to include susceptible species. However, the pathogen was highly virulent only to *A. virginica, Lalhyrus arboreus* L. and a few cultivars of *Pisum sativum* L.

The host range of *Phytophthora citrophthora* (Butl.) Butl. Included other than *Morrenia odorata* (H & A.) Lindl. Species (Ridings *et al.,* 1976). The host range included onion, watermelon, okra, tomato, carrot, English pea and cucumber.

Walker and Sciumbato (1979) studied the host range of *Alternaria macrospora.* Zimm., a potential bioherbicide of spurred anoda (*Anoda cristata* (L.) Schlect. The pathogen was inoculated to plants belonging to families of

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malvaceae, solanaceae, leguminaceae and graminae. It was noticed that the infection was restricted to plants in the malvaceae with spurred anoda being the most susceptible species. However, negligible damage was incited on cotton, hollyhock, prickly sida and velvet leaf.

Conway *et al.* (1977) employed a modified centrifungal (related plants) and variental (economic plants) strategy while conducting host specificity studies on over 85 plant species, which indicated the specificity of *Cercorpora rodmanii* Conway only to water hyacinth.

Host range studies of *Colletotrichum gloeosporioides* against *Clidemia hirta* (L.). D. Donof. confirmed the host specific nature of the pathogen. On other members of the family, *C. gloeosporioides* produced only appressoria on epidermis and further it failed to penetrate (Trujillo, 1986).

Host specificity of *Colletotrichum gloeosporioides* (Penz.) Sacc *malvae* was studied and found to be restricted to single family malvaceae except two other species.Safflower (*Carthamus tinctorius* L.) and white mustard (*Brassica hirta.* Moench.) on which it produced mild lesions (Mortenson, 1988).

Giannopolitis and Chrysayi (1989) isolated two species of *Septoria* and one species of *Phoma* from *Convolvulus arvensis* L. and they demonstrated the host specificity of the same. Both the organisms were host specific, however they caused mild lesions on two closely related plants i.e. *C. althaeoides* L. and *Calystegia sepium* L.

Host range study of *Fusarium pallidoroseum* a bioherbicide of *Parthenium hysterophorus*, on thirteen different crops revealed that, cowpea, cucumber, jowar and paddy were resistant, brinjal and lady's finger were moderately

resistant and chilli, cauliflower, coriander, maize, radish and tomato were susceptible (Kauraw and Bhan, 1993).

Elango *et al.* (1993) carried out host specificity screening trials using 16 local composite weeds related to *Chromolaena odorata.* The test indicated that, the rust *Cionothrix praelonga* was strictly host specific, while *Septoria ekmaniana*, a leaf spot pathogen showed a very restricted host range.

Shivas *et al.* (1994) carried out host specificity test of *Phomopsis emicis* Shivas a stem blight pathogen of *Emex australis* Steinh, a major annual weed of Australia. The test showed that, *P. emicis* caused leaf lesions and stem collapse only on *E. australis* and other two closely related Emex weeds.

Host range study of *Septoria passiflorae* Syd. isolated from Banana Poka vine *Passiflora tripartita* (Juss.) Poir. var. *tripartita* Holm - Nie. J & L in Hawaii forests demonstrated its specificity to two introduced weeds, *P. tripartita* var. *tripartita* and *P. foetida* L. The economic crops and other species in that experiment were immune (Trujillo *et al.,* 1994).

Host specificity of *Fusarium pallidoroseum* (Cooke) Sacc was studied by two methods viz., detached leaf technique and in vivo testing on intact plants. The test determined that, all the economic crops tested including species of asteraceae were resistant to infection and only the target weed parthenium was susceptible (Anon, 1998).

2.6 Inoculation techniques

Most of the mycoherbicides developed are foliar pathogens and the very common method of inoculation followed was spraying. Spraying of conidia or

conidia + mycelium through hand automizer was followed for inoculation (Walker and Sciumbato, 1979: Boyette *et al.,*1991a; Makowski, 1993).

Foliar inoculation technique was used to control field bind weed plants with *Phomopsis convolvulus.* Conidial suspension was prepared in deionized water with 0.1 % (w/v) gelatin solution and sprayed with a full cone nozzle until run off. Sprayed plants were placed in a dew chamber at 100 per cent R.H at 20°C in dark for 18 h for symptom expression (Morin *et al.,* (1989).

For knowing the best method of inoculation for the control of *Parthenium hysterophorus*, *Fusarium pallidoroseum* was inoculated by three different methods i.e., seed inoculation, soil inoculation and spray on the seed. All the three methods reduced the seed germination and growth as compared to the control. However, soil treatment was better as compared to seed treatment and spray on the seeds (Kauraw and Bhan, 1993).

2.6.1 Spore dose and vulnerable stage of the weed for infection

Standardization of spore dose and vulnerable weed stage are the factors of disease severity and mycoherbicidal efficacy. TeBeest *et al.* (1978) reported that, 100 per cent infection of *A. virginica* by *C.gloeosporioides f.* sp. *aeschynomene* occurred with spore concentration above 1×10^5 spores/ml at 28°C.

Alternaria macrospora controlled spurred anoda both in the green house and field when conidial suspension @ 1-5 x 10^5 spores/ml was sprayed (Walker, 1982). Morin *et al.,* (1989) opined that, foliar disease of *Convolvulus arvensis* caused by *Phomopsis convolvulus* was dependent on the amount of inoculum and inturn effectiveness of various inoculum density in killing the weed was related to plant growth stage. Weed seedlings at cotyledon stage

were severely injured and killed with 10^8 conidia /m^2. Young seedlings collapsed and died when inoculated with the same conidial density.

TeBeest (1991) demonstrated increased susceptibility of morning glory spp. to the rust *Coleosporium ipomoeae* after flowering and he stressed the necessity of testing at more than one developmental stage.

Makowski (1993) studied the effect of inoculum concentration and plant growth stage on anthracnose disease development of round leaved mallow (*Malva pusilla,* Smith) and velvetleaf (*Abutilon theophrasti* Medic). He recorded highest levels of control with an inoculum concentration of $2x10^6$ spores/ml for *M. pusilla* and $4x10^6$ spores/ml for *A. theophrasti.* All the growth stages of weeds were susceptible to *C. gloeosporioides* f.sp. malve. although younger seedlings were less susceptible than older plants.

Hoagland (1995) observed that, infection severity was proportional to the spore concentration. *Alternaria cassiae* J & K @ 10^6 spores/ml caused complete mortality of *Cassia alata* L. at 4-5 days after spraying. He also standardized the susceptible stage of the weed for infection and opined that, infection severity was inversely proportional to age of the plant. Young plants of 1-3 week old were the most crucial stage for infection, whereas older seedlings of 5-7 weeks escaped the infection. Klein and Auld (1995) obtained the best result when the mycoherbicide *Colletotrichum orbiculare* (Berk. et M) V. Arx. applied to young actively growing *Xanthium spinosum* L. plants during long dew periods. Further they assessed the influence of spore dose and water (carrier vol.) with/without oil on anthracnose incidence under field conditions. Spore dose of $2.5\ x10^{11}$, $5\ x10^{11}$ and $1x10^{12}$ conidia resulted in satisfactory control of the weed.

Deshphande *et al.,* (1997) isolated a blight pathogen *Alternaria alternata* from lower leaves of parthenium plants and identified that the seedlings at two leaf stage were highly susceptible. *A. alternata* @ $50x10^3$ spores/ml was able to kill the seedlings within six days of application.

Parthenium plants sprayed with *Fusarium pallidorseum* @ 10^{10} spores/ml showed highest necrotic leaf area (98.22%), where as the lowest (33.67%) was recorded on plants treated with 10^6 conidia/ml. However, optimum spore dose was 10^8 spores/ml, above which disease severity remained constant. Although, all the growth stages were susceptible to the pathogen, younger plants (3-5 and 6-9 leaf stage) were more susceptible than older plants (10-13 leaf stage) (Anon, 1998).

2.6.2 Histopathological studies

Very few studies have been made pertaining to histological responses of the weed to the pathogens. Studies on histopathology of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* on northern jointvetch revealed that, the pathogen penetrated the epidermis via appressoria and stem trichomes. Hyphae was noticed within epidermal, cortical, cambial vascular and pith ray tissues. In the infected cells the cytoplasm was disrupted and disorganized. (Te Beest et al., 1978).

Histopathological studies of *Botryosphaeria ribis* a bioherbicide of *Melaleuca quinquenervia* revealed that hyphal colonization was extensive in the cambium and phloem regions. Hyphae grew inter and intracellularly in the cortex, cambium, xylem and pith. Invasion and discoloration of phloem, cambium and xylem was observed. (Rayachhetry *et al.*, 1996).

2.7 Synergism/antagonism of pathogens

Crawley *et al.* (1985) studied the interaction of *Alternaria macrospora* Zimm and *Fusarium lateritium* Nees ex. Fr. on spurred anoda (*Anoda cristata* (L.) Schlecht). The seedlings even at older age showed 100 percent mortality by the interaction of *A. macrospora* and *F. lateritium*. Highest mortality was recorded, when *Alternaria* was inoculated five days before *Fusarium* inoculation.

Charudattan (1986) studied the interaction of *Colletotrichum dematium* f *sp.crotalariae* and *Fusarium udam f sp. crotalariae* to control showy Crotalaria. .Mortality of ground sel weed (*Senecio vulgaris* L.) infected by *Puccinia lagenophorae* Cooke was attributed to invasion of rust lesions by *Botrytis cinerea* Pers. Plants previously infected by *P. lagenophorae* died after inoculation with *Botrytis cinerea* (Hallett *et al.*, 1990).

Morin *et al.* (1993) studied the synergism between *Puccinia xanthii* Schw and *Cercospora orbiculare* on X*anthium occidentale* L. and observed that, necrotic lesion of *C. orbiculare* always occurred on or in proximity of leaf or stem lesions of *P. xanthii.*

2.8 Mass production of mycoherbicides and efficacy in the field

Boyette *et al.* (1979) reported that, for small scale mass production, V-8 vegetable juice or agar - agar cultures can be used to induce sporulation and obtain inoculum of several mycoherbicides.

Churchill (1982) studied mass production of mycoherbicides by employing two different modern fermentation techniques viz., solid culture fermentation and submerged liquid culture fermentation. He opined that, crude

agricultural products could be used in mass production of mycoherbicides. Boyette (1982) used oat seeds infested with *Fusarium solani* f. sp. *cucurbitae* Synd. Hans. to control Texas gourd *Cucurbita texana* (A.) Gray. through solid culture fermentation.

The commercially produced mycoherbicides COLLEGO AND DEVINE were both produced using submerged culture technique (Churchill, 1982; Boyette *et al.,* 1991b). Boyette *et al.* (1991b) reviewed the development and advancement in mycoherbicide production, formulation and application technology. They included one more fermentation technique i.e., combined solid substrate and submerged fermentation technique in addition to the earlier two techniques.

This new technique, 'combined solid substrate and submerged fermentation technique' was used to produce several mycoherbicides like *Alternaria macrospora* Zimm. against spurred anoda (Walker, 1980,1981) *Colletotrichum malvarum* (A. Braun & Casp.) against prickly sida (*Sida spinosa* L.) and *Fusarium lateritum* south worth, a mycoherbicide for spurred anoda, prickly sida and velvet leaf (Walker, 1981). Each gram of culture medium of *A. macrospora* produced a dry weight of 4 g of spores and spore yields were 1×10^5 spores/g of dried mycelium (Walker, 1981).

Modifications of this methodology were made to produce spores of *Alternaria cassiae* against sickle pod and each gram of spores contained $\simeq 1 \times 10^8$ spores (Walker and Riley, 1982). This technique was also used to produce spores of *A. crassa* for jimson weed (*Datura stramonium* L.) control. (Boyette *et al.,* 1986). *Alternaria helianthi* (Hansq.) T & N against Cocklebur (*Xanthium strumarium* L.) and wild sunflower (*Helianthus annus* L.) (Quimby, 1989).

Stowell (1991) gave a detailed picture of three different techniques developed by considering submerged fermentation' for production of bioherbicides. Later, he patented these three techniques.

Conway *et al.* (1978) gave the process diagram for production of *cercospora rodmanii, a* mycoherbicide against water hyacinth. After the harvest of mycelium, it was formulated into either granules or sprayable material.

Other flow diagrams were developed for production of *C. gloeosporioides f.sp. aeschynomene* (Daniel *et al.,* 1974), *C. malvarum* against prickly sida, velvet leaf and other mallow species (Templeton, 1976) and *Alternaria cassiae* against sicklepod (Walker, 1983); *Fusarium lateritium* against prickly sida, velvet leaf and spurred anoda (Walker, 1983).

In field trials conducted during 1985 to 1987 at Arkansas, jimson weed was successfully controlled with conidial application of 1.34 kg/ha of *A. crassa.* Conidial formulations were superior over mycelial formulations. But under favourable environmental conditions both the formulations had similar effect (Boyette *et al.,* 1991a).

2.9 Physiological and biochemical changes in host-pathogen interactions

Hoagland (1990) studied the physiological changes in sickle pod (*Cassia obtusifolia* L.) due to the infection of *Alternaria cassiae* , He recorded the increased phenyl alanine ammonia lyase (PAL) activity in the infected plants. Similarly, levels of PAL products were also raised in shoots of infected plants and increase in concentration was related to the appearance of leaf lesions and necrotic spots during host-parasite interaction.

Yang and Guo (1991) studied the effect of *Mycovellosiella eupatorii - odorata* on the growth and physiology of *Eupatorium adenophorum*. Photosynthetic rates of 39.60 percent and 68.60 percent and transpiration rates 62.70 percent and 67.60 percent higher respectively than that of control. Plant height, leaf number and flower number of infected plants were also lower than that of control.

Kempenaar *et al.* (1996) studied the effects of *Ascochyta caulina* (P. Karst.) A & V. on photo synthesis of leaves of *Chenopodium album* L. He reported that, net photosynthetic rate was decreased with increasing proportion of necrotic area.

Tang *et al.* (1996) studied the host-pathogen interaction in *Albugo candida - Arabidopsis thaliana* L. system. They recorded decreased photo synthesis and chlorophyll in affected plants. In affected leaves, both soluble carbohydrates and starch were accumulated.

Shabana *et al.* (1996) recorded the lowest levels of pigments, carbohydrates and relative water content in water hyacinth infected with *Alternaria eichhorniae.* Infection led to an increase in total phenol content of leaves compared to untreated control.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Laboratory experiments were conducted at the Plant Pathology Laboratory and glass house, Department of Plant Pathology, College of Agriculture, Dharwad during 1995-97 and the field experiments were conducted at the Farm Forestry Station, University of Agricultural Sciences, Dharwad from 1995-97 to study the management of *Chromolaena odorata* through use of mycoherbicides. The relevant details of the experimental procedures followed and materials used for laboratory and field experiments are presented in this chapter.

3.1 Laboratory experiments

3.1.1 Glassware and cleaning

In all the experimental studies, Borosil glassware were used. The glass ware were kept in the cleaning solution containing 60 g potassium dichromate ($K_2Cr_2O_7$), 60 ml concentrated sulphuric acid (H_2SO_4) in 1000 ml of water for 24 h and they were washed with Vim powder by cleaning in tap water.

3.1.2 Sterilization

All glassware were sterilized in autoclave at 1.11 kg pressure/sq.cm for 20 minutes. The solid and liquid media were sterilized at 1.11kg pressure/sq. cm for 15 minutes. Soil used for the experimental purpose was sterilized in an autoclave for 2 h at 1.33 kg pressure per sq.cm.

3.2 Field experiments

The field experiments were conducted on naturally grown Eupatorium at the Farm Forestry Department, University of Agricultural Sciences, Dharwad

during 1995-97. Dharwad is situated at 15°2' North latitude and 75°7' East longitude at an altitude of 678 m. above the mean sea level.

Weather Parameters

The weather data during the experimental period (96-97) presented in the Appendix I indicated that, the total rainfall was 801 mm. The maximum rainfall received during June (130 mm) and the minimum in March (5.0 mm). The maximum temperature at Dharwad varied from 37.4°C (May) whereas the minimum temperature ranged from 13.5°C to 21.5°C during December to June respectively. The relative humidity however, was maximum during July (87%) and minimum during April (60%).

3.3 *Survey for Eupatorium Diseases*

A survey was conducted in different parts of Eupatorium growing areas of Karnataka during 1995-96 (July'95 to Feb.'96) at different weed growth stages. Eupatorium is endemic in the following districts of Karnataka.

1. Dharwad 2. Belgaum 3. Shimoga 4. Uttar Kannada

An intensive survey was carried out in above district during vegetative as well as flowering stages of the weed. In each district, three talukas were selected and diseased samples of eupatorium covering field, road side, plantations and forest areas were collected and the symptoms were described. After recording symptoms the specimens were preserved in plant press for further studies.

3.3.1 Isolation of Fungi

The fungi were isolated from diseased parts of weed by following standard tissue isolation procedure. The diseased plant parts were cut into small bits and washed thoroughly in water. The bits were surface sterilized in 1:1000 mercuric chloride ($HgCl_2$) solution for 30 seconds and 15-20 seconds for leaf and flower parts respectively. The bits were washed thrice in sterile distilled water to remove the traces of mercuric chloride, if any, and then transferred aseptically to potato dextrose agar slants. Inoculated tubes were incubated at room temperature of 27± 1°C for seven days to obtain good growth of the fungus.

3.3.1.1 Hyphal tip isolation

This method is followed for maintaining pure culture of *Bipolaris* sp. since, it is known to be highly heterozygous. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on water agar plates and observed for spore under microscope. Single isolated spore was then marked with ink. Such plates were incubated at 27 ± 1 °C and periodically observed under the microscope for germination of spores. Hypha coming from the cell of a spore was traced and marked with the ink. Then the tip of the hypha was cut, transferred to the PDA slants and incubated at 27 ± 1 °C for one week.

Subculturing was done for every 15 days. The fungal isolates were subcultured on potato dextrose agar slants and incubated at 27± 1°C for a week. After incubation the tubes were preserved in a refrigerator at 4°C which were used for further studies.

3.3.2 Identification of fungi

For identification of fungi, the isolates were grown on potato dextrose agar (PDA) slants and sent to Agarkar Research Institute, Maharashtra Association for cultivation of Sciences, Pune (Maharashtra)

3.3.3 Pathogenicity Test

Pathogenicity test was carried out under glass house conditions. Eupatorium plants were grown in 50 x 30 cm diameter pots filled with five kg of sterilized soil. All the fungal pathogens isolated from different localities were grown on potato dextrose agar medium in Petriplates for 15 days. After incubation period the culture plates were flooded with 15 ml sterile distilled water. The growth was macerated and thoroughly mixed with water. This semi solid suspension containing mycelium as well as spores was sprayed on two months old eupatorium plants until runoff. Control plants were sprayed with sterile water and all the treatments were replicated twice. Both inoculated and uninoculated plants were covered with punched polythene bags and high humidity was maintained. Observations were made for the typical symptoms. Soon after the appearance of symptoms the organism was reisolated from leaves / inflorescence and the culture thus obtained was compared with the original culture for confirmation.

Studies were concentrated only on highly virulent and potential pathogen of *C. odorata.* Hence, further experiments were envisaged based on two criteria to select highly virulent pathogens.

a) Potentiality of pathogens to cause severe necrosis/sooty mold (*In vivo* criteria).

b) Growth and sporulation capacity of pathogens on different nutrient media (*in vitro* criteria)

Three features *viz.*, degree of damage, profuse growth and sporulation capacity will make a pathogen to be a fit mycoherbicide.

First criterion was accomplished by screening the different pathogenic isolates under glass house conditions.

3.4 Screening of different pathogenic isolates for their efficacy in inducing disease on *Chromolaena odorata*

This experiment was conducted to select only potential isolates among the different pathogenic isolates of eupatorium. The plants were grown in pots and culture suspension was inoculated as explained in pathogenicity test

Observations on per cent disease index were calculated at 10 days and 15 days after spraying. The pathogens were screened and selected based on their ability to cause disease (Leaf spots/black mould) on eupatorium. For the assessment of disease severity, a scale was also developed.

Disease assessment scale (Plate 1)

0-5 scale was used to calculate the severity of the disease.

Grade	Description
0	Plant completely free from infection
1	Very little infection, with a few small lesions 1-5 per cent of the leaf area affected.
2	6-20 per cent of the leaf area affected

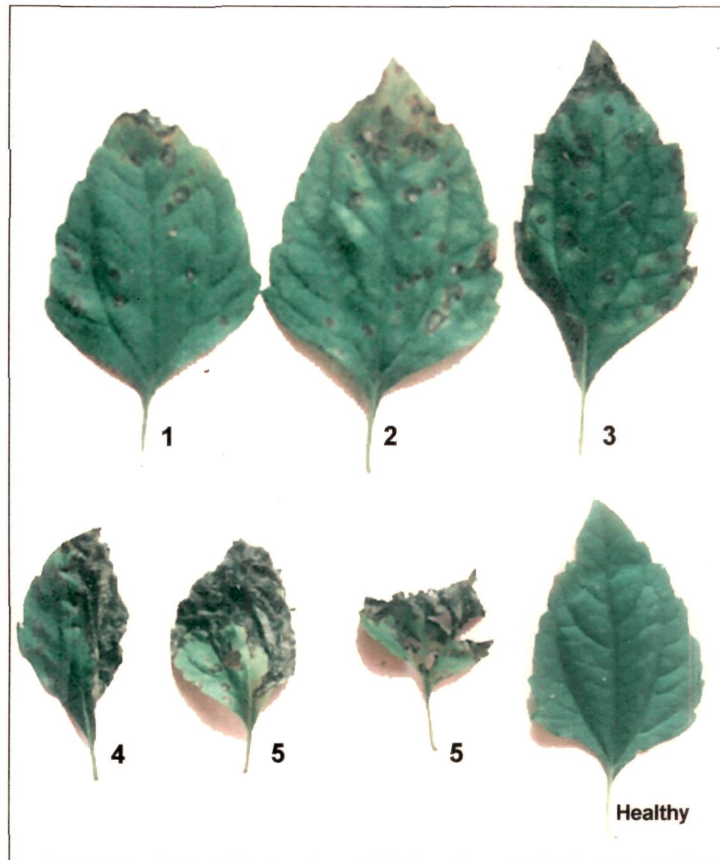


Plate 1 : Eupatorium leaves indicating disease assessment scale

3	21-40 per cent leaf area is affected either by marginal necrosis or by lesions.
4.	41-60% of the leaf lamina may be covered with the disease.
5.	More than 60% of leaf area affected. Lesions further increase in size and number. The cracking and shedding of the necrotic lesions, giving an irregular shape to the leaf. Leaves may become brittle and defoliation is common.

The per cent disease index was calculated as per the formula proposed by Wheeler (1969).

$$\text{Per cent disease Index} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves assessed}} \times \frac{100}{\text{Maximum disease grade}}$$

3.5 Cultural and morphological characters of potential pathogens

This experiment was carried out to screen and select the highly virulent pathogens among the several selected isolates and find out the best medium for growth and sporulation of the pathogens. The medium capable of yielding maximum growth and high fecundity was further selected as culture medium for mass multiplication of the pathogens. The cultural characters of the pathogens were studied with reference to colony characters, colour of the mycelium, type of growth, growth rate and sporulation capacity on synthetic and non synthetic media.

The composition and the procedures for preparation of the media used in this experiment were followed as explained by Tuite (1969). The chemical composition of each medium is as follows.

Synthetic medium

1. **Czapek's medium**

Sucrose ($C_{12}H_{22}O_{11}$)	:	30.00 g
Sodium nitrate (Na NO_3)	:	2.00 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	:	0.05 g
Potassium chloride (KCl)	:	0.50 g
Ferrous sulphate ($FeSO_4$ $7H_2O$)	:	0.01 g
Dipotassium hydrogen phosphate (K_2HPO_4)	:	1.00 g
Agar-agar	:	20.00 g

2. **Czapek's Dox medium**

Sucrose ($C_{12}H_{22}O_{11}$)	:	30.00 g
Sodium nitrate (Na NO_3)	:	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	1.00 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	:	0.50 g
Potassium chloride (KCl)	:	0.50 g
Ferrous sulphate ($FeSO_4$ $7H_2O$)	:	0.01 g
Agar-agar	:	20.00 g

3. **Richards's medium**

Sucrose ($C_{12}H_{22}{}^{o}{}_{11}$)	:	50.00 g
Potassium nitrate (KNO_3)	:	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	5.00 g
Magnesium sulphate (Mg SO_4 $7H_2O$)	:	2.50 g
Ferric chloride ($Fecl_3$ $6H_2O$)	:	0.02 g
Agar - agar	:	20.00 g

All the ingredients except potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 450 ml of distilled water. Agar was melted in 500 ml distilled water and mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml distilled water. Both the solutions sterilized separately and mixed together at the time of pouring to Petri plates.

4. Sabouraud's dextrose agar

Dextrose ($C_6H_{12}O_6$)	:	40.00 g
Neo peptone	:	10.00 g
Agar-agar	:	20.00 g

5. Tochina's medium

Peptone	:	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	0.50 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	:	0.25 g
Maltose ($C_{12}H_{22}O_{11}$)	:	10.00 g
Agar-agar	:	20.00 g

All the ingredients were dissolved in 400 ml of distilled water and the agar was dissolved separately in 50 ml distilled water and mixed with the above solution. The volume was made up to one litre. The medium was sterilized at 1.1 kg/cm^2 pressure for 15 minutes.

Non - Synthetic media

Eupatorium leaf Extract Agar

Eupatorium leaf bits	:	200.00 g
Agar-agar	:	20.00 g
Distilled water	:	1000.00 ml

Eupatorium leaf Extract Agar + 1% Sucrose

Eupatorium leaf bits	:	200.00 g
Sucrose	:	10.00 g
Agar-agar	:	20.00 g
Distilled water	:	1000.00 g

Oat meal agar (Johnson and Curl, 1972)

Oat meal	:	17.00 g
Yeast extract	:	1.00 g
Agar-agar	:	17.00 g

Potato Dextrose Agar (PDA)

Peeled potato	:	200.00 g
Dextrose ($C_6H_{12}O_6$ H_2O)	:	20.00 g
Agar-agar	:	17.00 g
Distilled water	:	1000.00 ml (to make up the volume)

Eupatorium leaf extract agar medium was prepared by boiling 200.00 g of the eupatorium leaf bits in 500 ml of water for 10 minutes and filtered through a muslin cloth. Twenty gram of agar was melted separately in remaining 500 ml of water. Both the solutions were mixed and the volume was made up to 1000 ml.

Fifteen ml of each of the medium was poured in to each of 90 mm sterilized Petriplates. Inoculation was made by transferring the five mm disc of mycelial mat, taken from the periphery of seven day old culture of each of the seven isolates. Each treatment was replicated thrice.

The plates were incubated at 27 ± 1°C. An observation on colony radial growth was taken when the maximum growth was attained in any one of the media tested. Other cultural characters viz., rate of growth, variation in topography (elevation), colony characters and sporulation were also recorded.

Sporulation

The sporulation capacity of each isolate on different media was assessed by microscopic observations. A loopful of culture was transferred to a clean slide and mixed well with lactophenol and a cover slip was placed on it. The rate of sporulation was recorded in five different microscopic fields.

Based on this experiment, the pathogens were screened and selected as potential pathogens which were used in further experiments.

Rate of Sporulation	No. of spores/microscopic field
Excellent	Above 70
Good	50-70
Fair	20-50
Poor	< 20
Nil	0

3.6 Growth phase studies of potential pathogens

Thirty ml of the potato dextrose broth was added in to each of the 150 ml conical flask and sterilized. The flasks were then inoculated with five mm mycelial discs obtained from the actively growing colony of each of the pathogen. The inoculated flasks were incubated at 27± 1°C. The growth in the flask was harvested after 4,6,8,10,12,14,16,18 and 20 days of inoculation. The cultures were filtered through previously weighed whatman No.1 filter paper which were dried to a constant weight at 60°C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with distilled water to get rid off the salts likely to be associated with the mycelial mats. The filter paper along with the mycelial mats were dried to a constant weight at 60°C in an electric over for 24 h and then cooled in a desiccator,. Finally weight of dry mycelial mats were recorded.

3.7 Growth studies in liquid media

The composition and preparation of different liquid media used, were the same as that of solid media except that agar - agar was not added. The flasks containing 30 ml of media were inoculated with inoculum of each pathogen as described earlier and were incubated at 27± 1°C for 12 days and 10 days for *A. alternata* and *C. gloeosporioides*, *A. pullulans,* respectively. The mycelial growth was harvested and dry mycelial weights were obtained. The best medium was found out and used as basal medium for further studies.

3.8 Physiological studies of potential pathogens

3.8.1 Effect of different temperature levels on the growth of potential pathogens

Sabouraud's liquid medium was used as a basal medium in this experiment. The pH of the medium was adjusted to two different levels viz., 5.50 for *A. alternata,* 6.0 for *C. 'gloeosporioides* and *A. pullulans,* since maximum growth of pathogens was obtained at these pH levels. Thirty ml of Sabouraud's medium was dispensed into each of 150 ml flasks and sterilized. Each flask was inoculated with inoculum as described earlier and incubated for 12 days and 10 days for best growth of *A. alternata* and *C. gloeosporioides, A. pullulans* respectively in incubators adjusted to the required temperature levels. The different temperature levels tested were *viz.,* 0°C, 5°C ,10°C, 15°C, 20°C, 25°C,30°C, 35°C,40°C & 45°C . Each treatment was replicated thrice and mycelial growth was harvested and dry mycelial weights were recorded.

3.8.2 Effect of different pH levels on growth of potential pathogens

Sabouraud's solution was the liquid medium used in this experiment. pH of the medium was adjusted by adding 0.1 N alkali (NaOH) or acid (Hcl). Reaction of the medium was adjusted to the desired pH by using dihydrogen PO_4 citric acid buffer according to the schedule of Vogel (1951). pH of the medium was adjusted to pH, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Each of the isolates was inoculated in to 20 ml basal medium and each treatment was replicated thrice. The flasks were incubated at 27 ± 1°C for 12 days and 10 days for *A. alternata* and *C. gloeosporioides, A. pullulans,* respectively. The best pH was found out and used in further studies.

3.9 Nutritional and biochemical studies of potential pathogens

3.9.1 Effect of different carbon sources on the growth of the potential pathogens

For carbon requirement studies, Sabouraud's medium was taken as a basal medium. Different carbon compounds were added on the basis of their molecular weight so as to provide an equal amount of carbon as was of dextrose present in Sabouraud's medium. Carbon compounds used in the study were glucose, dextrose, fructose, mannitol, sucrose, lactose and citric acid.

All the sugars dissolved properly in separate flasks and then sterilized at 1.11 kg pressure per sq. cm. for 15 minutes. Each treatment was replicated, thrice. The flasks were inoculated as described earlier and incubated at 27 ± 1°C for 12 days and 10 days for *A. alternata* and *C. gloeosporioides, A. pullulans,* respectively. The mycelial growth was harvested and dry myeclial weights were recorded. The best carbon source was found out and used for further studies.

3.9.2 Effect of different nitrogen sources on the growth of the potential pathogens

For nitrogen requirement studies, Richards's medium was taken as a basal medium. Different nitrogen sources used in the experiment were *viz.,* L - asparagine, L - methionine, L - tyrosine, urea, ammonium nitrate, peptone, potassium nitrate and sodium nitrate.

The quantity of nitrogen source added was determined on the basis of their molecular weight, so as to provide an equivalent amount of nitrogen as was of potassium nitrate in the basal medium. The flasks were inoculated as described earlier and three replications were maintained for each treatment. Inoculated flasks were incubated at 27 ± 1°C for 12 days and 10 days for *A. alternata, C. gloeosporioides* and *A. pullulans,* respectively. The mycelial growth was harvested and dry mycelial weights were recorded.

3.10 Effect of culture filtrate of potential pathogens on tomato and eupatorium cuttings

The potential pathogens were grown on potato dextrose agar medium. A mycelial disc of five mm diameter was inoculated in to 100 ml flasks containing 30 ml of Richards's medium. The flasks were incubated at 27 ± 1°C for ten days. The culture filtrate of each pathogen was obtained by filtering through a Whatman no. 1 filter paper. The filtrate was diluted to 1:1 by adding sterile distilled water.

Actively growing young stem cuttings of tomato and eupatorium were dipped in test tubes containing the culture filtrate. A control was maintained by dipping tomato and eupatorium stem cuttings in sterile water as well as in Richards's medium such tubes were incubated on laboratory working table. Observations on drooping, necrosis and complete wilting of cuttings were recorded for each of organisms after 24 h and 48 h of incubation.

3.11 Host range studies

A critical consideration in the development of a mycoherbicide is the determination of host range. Irrespective of potential damage on target weeds

the safety of non target cultivated and wild plants must be ensured prior to use of mycoherbicide.

In this experiment the plant species were selected based on modified centrifugal (related plants) and variental (economic plants) strategy (Conway *et al.*, 1977). The plant species evaluated were grouped into two categories.

Category A: (Weeds) Parthenium, *Lantana camera*

Category B: Field crops, Plantation and Forest Plants, where eupatorium is always associated.

Field Crops : Sunflower, cotton, paddy and cowpea

Plantation crops : Arecanut, coconut, pepper, beetle vine, banana, cardamom, cocoa, cashew and coffee

Forest trees : Eucalyptus, teak and bamboo

Host range of potential pathogens of eupatorium was tested by employing 'Detached leaf technique'. Each detached leaf of the test plants was washed in tap water and then in sterile water. Five mm culture disc of each pathogen was inoculated on leaf lamina at three points in a triangular manner. Inoculated leaves were kept in Petriplates of 15 cm containing moist filter paper and 5 ml of 1 per cent sucrose solution. Care was taken to see that, only leaf petiole was immersed in 1% sucrose solution in order to preserve the colour of leaves. For bigger sized inoculated leaves, polythene bags were used to cover them. Suitable controls and replications were maintained. All the inoculated leaves were examined daily upto a week for the appearance of

symptom. The leaves showing typical symptoms were examined microscopically and following observations were recorded.

Observations : 1) Spore germination, 2) Colonization, 3) Pathogenesis, 4) Symptom expression, 5) Infectivity level and 6) Dissemination

3.12 Survey and surveillance for potential pathogens of *Chromolaena odorata*

Survey and Surveillance was conducted during 1996-97 to confirm the endemic nature of the potential pathogens of eupatorium.

During this study, Yellapur, Dharwad, Prabhunagar (Forest Research Station) and Mundagod forest areas were surveyed at vegetative stage as well as in reproductive stages, to know prevalence of potential pathogens. From each area, 25 plants were randomly selected and from each plant disease leaves / flowers with leaf spots/black mould were selected. These samples were further, microscopically examined and isolations were made and per cent recovery of potential pathogen was recorded.

3.13 Inoculation techniques

Method of inoculation for each pathogen may vary and pathogens may able to cause maximum infection only when they are inoculated by the specific method. Hence, different inoculation techniques were evaluated to find out which method is best suited for a pathogen.

Following inoculation techniques were evaluated to standardize the inoculation method for potential pathogens under glasshouse conditions.

a. Seed inoculation

Eupatorium seeds (Cypsella) were rolled on heavily sporulating culture on potato dextrose agar and sown in Petriplates containing three layers of moist filter papers. Each treatment was replicated five times. Seeds without inoculation served as control. Observation on pre and post emergence mortality was recorded.

b. Soil inoculation

Seedlings were raised in pots of 10 x 15cm size which contained 500 g sterilized soil. The pathogens were grown on PDA and the culture was blended. The blended culture contained spores as well as mycelium and it was drenched to soil at 10^{15} spores/ml.

c. Stem inoculation

The culture suspension of each of the pathogen was prepared and was injected to two months old weed stem with a hypodermic syringe at 10^{16} spores per ml.

d. Foliage inoculation

First the plants were sprayed with sterile water to provide high humidity. The spore suspension of each pathogen was prepared in sterile water and sprayed on foliage at two months old plant at 10^{16} spores per ml using an atomizer until runoff and then covered with polythene bags to retain high humidity. Control plants were sprayed only with sterile water. Observations were recorded at regular intervals for symptom expression.

e. **Flower inoculation**

To facilitate quick germination of spores and disease initiation, first the flowers were sprayed with sterile water. Later culture suspension of each pathogen was sprayed on just opening flower at 10^{15} spores per ml. All the inoculated flowers were covered in polythene bags to maintain high humidity. The flowers receiving sterile water spray were considered as control. Observations were made for specific symptom expression.

Inoculation of pathogen by standard inoculation technique should follow the susceptible age of the weed at optimum spore dose. When all these three conditions coincide, there will be maximum disease and control of the weed.

Following observations were recorded

1. Concentration of spores required for infection and standardization of spores for maximum infection.
2. Standardization of susceptible stage for maximum infection
3. Pathogenesis and period required for symptom expression
4. Histological changes due to host pathogen interaction

3.13.1 Standardization of spore dose for maximum infection

The experiment was carried out under glass house conditions.

Inoculum production

All the potential pathogen were cultured on PDA medium and allowed to grow for ten days. Spores / conidial suspensions were obtained by flushing the

surface of sporulating cultures with 15 ml of sterilized distilled water. Further the spore suspension was adjusted to varying levels with the help of a haemocytometer. The different spore `concentration tested were viz., 0, 10^2 10^4, 10^6, 10^8, 10^{10}, 10^{12}, 10^{14}, 10^{16}, 10^{18} and 10^{20} spores per ml. The suspension containing different spore dose was sprayed individually on two months old eupatorium plants. High humidity was maintained on the foliage as well as blossom for 24 to 48 h after inoculation

Degree of infection was recorded soon after the disease symptoms were observed. Spore load of each pathogen was standardized based on degree of infection. In further studies standardized spore load was used. The per cent disease index was calculated by using the formula of Wheeler (1969).

3.13.2 Identification of vulnerable stage of the weed for maximum infection

The experiment was carried out under glasshouse condition. The cypsella separated from the capitula were subjected to germination in petridishes on top of double layered filter paper moistened with distilled water. After germination, they were transferred carefully to pots of size 10 x 15 cm containing 500 gm sterilized soil. The pots were irrigated optimally to facilitate vigorous growth of seedlings.

Spore suspension with optimum spore load as standardized in earlier experiment as sprayed over weed plants of one month, two months, four months, five months old and plants at different flowering stages (Bud initiation, Flower initiation, Flower opening and full bloom) until run off. Four replications were maintained for each treatment.

Susceptible stage for maximum infection was identified based on degree of disease severity caused by the pathogen. Observations on per cent necrosis / disease was calculated by using the formula of Wheeler (1969).

3.13.3 Histopathological studies

The standard procedure of microtomy technique was followed for the study.

Sampling

The leaves were collected randomly from healthy and diseased plants and they were washed thoroughly in tap water to remove all the dusts present on them before fixing.

Fixation

The leaves collected were cut into pieces of 2.0 cm length. Then the leaves were fixed for 24 h in standard FAA fixative (Formalin: Aceticacid ; Alcohol) in the ratio of 5 ml of formalin: 5 ml of acetic acid; 90 ml of 70% alcohol.

Dehydration

Fixed leaves were washed thoroughly with 70 per cent alcohol and dehydrated using ethanol (70%, 90% and absolute alcohol respectively) and n-butanol in combination with alcohol in the ratio of 1:3, 1:1, 3:1 and absolute butanol leaving the material in each grade for a period of three hours.

Paraffin infiltration and embedding:

Paraffin wax (58-60°C melting point) was used for infiltration and embedding purpose. Small chips of paraffin wax were added successively to the medium of pure n-butanol containing dehydrated sample, until the medium reached a saturation point at room temperature. The specimen were kept in an oven maintained at 60°C. Subsequent changes with fresh molten paraffin were given at every four hour interval to replace even the last traces of butanol with paraffin. The specimen were further embedded in paraffin wax (60°C) employing paper boat technique (Jensen, 1962). The paper boats were coated priorly with glycerine.

Microtoming and affixing the sections:

Uniformly thin sections of 10 μm thickness were cut with the help of Erma type rotary microtome. The paraffin ribbons were cut into convenient lengths with the help of a blade and placed on the pre-cleaned slides flooded with adhesive solution. One per cent gelatin was used as an adhesive (Jensen, 1962). The slides were then warmed over a warming plate maintained at nearly 45°C to facilitate flattening and stretching of the ribbon. The excess adhesive material was removed carefully without disturbing the sections and slides were later dried in a dust free environment for 72 h under room temperature.

Staining: (Safranine - Fast green stain)

For histopathological studies, the following staining procedure was followed.

1. The paraffin embedded sections were deparaffinised by passing the slides through xylene for five minutes. The sections were transferred to 1:1 mixture of xylene and absolute alcohol for another five minutes.

2. Sections were partially hydrated by passing through a series of alcohol of decreasing concentration i.e. absolute, 95 per cent, 70 per cent and 50 per cent (5 minutes in each).

3. The sections were stained with 1 per cent safranin (1 g saffranine in 100 ml absolute alcohol and diluted to 1:1 with distilled water) for one hour.

4. The slides were washed thoroughly in water, passed quickly through acidified 70 per cent alcohol to destain the excess stain and then passed rapidly through 95 per cent absolute alcohol.

5. Counter staining was done with fast green (0.5 gm of fast green in 50 ml clove oil and 50 ml alcohol) for 1 to 5 minutes.

6. The fast green was differentiated by placing in 50 per cent clove oil, 25 per cent alcohol and 25 per cent xylene.

7. The sections were placed in xylene, making three changes of at least 15 minutes each and finally mounted with coverslip using DPX mount.

3.14 Interaction effect of potential pathogens on eupatorium

This study was initiated to evaluate the different combinations of potential pathogens and their synergism / antagonism to cause disease on the weed. The experiment was carried out under glasshouse conditions. The weed plants were grown in pots of 50 x 30 cm size containing five kg sterilized soil. The pots were watered regularly to maintain optimum moisture levels.

All the potential pathogens were grown on PDA medium contained in Petriplates. Sterile distilled water was used to harvest the spores as inoculum. The spore load (spores/ml) of each pathogen required to initiate maximum disease on the weed was adjusted with the help of a haemacytometer.

The pathogens were inoculated spraying individually and in all possible combinations on weed. Optimum spore concentration of each pathogen was maintained during spraying. The plants were sprayed up to runoff with an automizer and covered with punched polythene bags. Each treatment was replicated thrice. Inoculated plants were incubated for three days and after three days the polythene bags were removed. Observations on per cent disease index was calculated using the formula proposed Wheeler (1969) and no of days required for disease symptom expression were also recorded

3.15 Mass production and field efficacy of pathogens

Different substrates were assayed for mass production of pathogens. The substrates tested were *viz.*, eupatorium leaf bits, eupatorium leaf extract + 1 per cent sucrose and potato dextrose broth. Based on sporulation rate favoured by each medium on each pathogen, substrate was selected for mass production.

Sporulation rate	No.of spores/microscopic field (10 x magnification)
Excellent (+ + + +)	> 70
Good (+ + +)	50 - 70
Fair (+ +)	20-50

There is sufficient literature available on the use of plant pathogens for weed control. However, if widespread control of weeds with plant pathogens

is to be accomplished, it is necessary to mass produce potential pathogens either in form of spores or other infectious inocula. The viable, infection inocula must be produced rapidly in an inexpensive medium and recoverable in an efficient and reproducible manner.

Stock culture

The stock culture used in mass productivity programme was agar slants (PDA) stored in defreezer at -4°C.

Selection of culture medium

The first step in production of microorganisms is the screening of different agar media and selection of superior medium which stimulates high inocula production. The procedures were same as explained in cultural and morphological studies. Based on these results, a medium was selected as 'seed medium' and used in further studies.

Mass culturing of mycoherbicides for large scale use

Two different techniques were adopted to produce mycoherbicides in large scale *viz.*,

1) Solid substrate Fermentation

2) Submerged culture Fermentation

3.15.1 Solid substrate fermentation

The solid substrate selected for mass multiplication was eupatorium leaf bits. Healthy leaves from eupatorium plant were collected and cut in to small bits (2-3 cm) and filled in polythene bags of one kg capacity and sterilized in an

autoclave at 1.33 kg pressure/sq.cm for 25 minutes. Such sterilized polythene bags were inoculated and incubated for 20 days for the maximum colonization and sporulation.

After 20 days, the leaf bits, which turned to jet black due to heavy sporulation, were mixed with water. The mixture was blended vigorously to make a spore suspension. Standard spore load was maintained and the suspension was used for spraying in the field after diluting with distilled water.

3.15.2 Submerged culture fermentation

The fungus that sporulated heavily in liquid culture can be multiplied by submerged culture fermentation method.

The infective propagules (spores + mycelium) of the pathogen were inoculated to potato dextrose broth in a series of 1000 ml conical flasks. Optimum pH and temperature of the medium were maintained. The inoculated flasks were incubated for 15 days. To get uniform growth and the maximum sporulation, the flasks were agitated for 5-10 min. in an agitator once in a week. After incubation, the growth was collected, blended and homogenised in the suspension. The homogenised broth was either directly used or filtered before diluting with the universal carrier 'water'. Then the suspension was sprayed on weed plants with a standardized spore load.

Precautions

1) To maintain high humidity, the weed plants to be inoculated were first sprayed with water.

2) After spraying, humid condition was maintained at least for 48 h to facilitate quick spore germination and spread of *A. pullulans*.

3) Spraying was carried out during evening hours.

3.15.3 Influence of spray additives on disease development

Mass produced pathogens were diluted with water and obtained in the form of suspension. The spore load of each pathogen was maintained while spraying. Effect of three per cent sucrose as a spray additive on disease development was studied in comparison with water.

The experiment was conducted on naturally grown eupatorium plants in Farm Forestry Station. The plants were selected randomly and sprayed with and without spray additive (3% sucrose) in spore suspension. Influence of spray additive on disease advancement was recorded.

3.16 Field evaluation of individual and combination of potential pathogens on eupatorium

The experiment was designed to find out the effect of spraying of individual and combination of pathogens on seedling mortality and seed viability of eupatorium weed.

Selection of Experimental Site

A field experiment was conducted on the naturally grown eupatorium weed at the Farm Forestry Station, University of Agricultural Sciences, Dharwad during 1996-97 (July 96 - Feb 97) (Plate 2).

Design and Layout : The experiment was designed in Randomized Block Design with three replications. The plot size was 2.0 x 2.0 m for each treatment.

Treatment details:
1. *Alternaria alternata* (Foliar spray)
2. *Colletotrichum gloeosporioides* (Foliar spray)
3. *Aureobasidium pullulans* (Spray on Flowers)

Plate 2. General view of the experiment site

4. *A. alternata* + *C. gloeosporioides* (Foliar spray)

5. *C. gloeosporioides* + *A. pullulans* (Foliar spray)

6. *A. alternata* + *A. pullulans* (Foliar spray)

7. *A alternata* + *C. gloeosporioides* + *A. pullulans*(Foliar spray)

8. Control (water + 3% sucrose) (Spray on Flowers and foliage)

The concentration of pathogens (spores/ml) required for maximum infection was maintained while inoculation. Spraying was carried out during evening time and 48 h dew period was maintained for treatments receiving *A. pullulans.*

Five plants in each treatment were selected randomly after spraying, to study different aspects viz.,

a) Per cent disease index (Necrosis / leaf spots / black mould).

b) Time requirement to develop epiphytotic conditions.

c) Host-pathogen interactions : Physiological, Biochemical and Bio physical changes in the weed due to host - pathogen interaction.

3.16.1 Per cent disease index (Necrosis / leaf spots / black mould)

The per cent disease index was calculated as per the formula proposed by Wheeler (1969).

3.16.2 Physiological, Biochemical and Biophysical changes in the weed due to host - pathogen interactions

3.16.2.1 Estimation of Chlorophyll Content

Total Chlorophyll content was determined by the method of Arnon (1949) at 7, 14 and 21 days after spraying the pathogen.

Fresh leaves of healthy and diseased plants were brought from the field in an ice box and cut in to small pieces. Known amount of fresh leaf (100 mg) was weighed from each treatment and homogenised with acetone. The extract was filtered through Whatman no.1 filter paper and washed twice with 80 per cent acetone. The final volume of the extract was made up to 25 ml. The absorbance of the extract was read at 645 and 663 nm in a Spectrophotometer (Systromics model, CL-54) against 80 per cent acetone.

The total Chlorophyll content was calculated by using the following formula and expressed in mg g' fresh weight.

$$\text{Total Chlorophyll} = \frac{(20.2 \times A_{645}) + (8.02 \times A_{663}) \times V \times D}{a \times 1000 \times w}$$

A = Absorbance at specific wavelengths (645 and 663 nm)

D = Dilution factor

V = Final volume of the Chlorophyll extract

W = Fresh weight of the Sample (g)

a = Path length of light (1cm)

3.16.2.2 Relative water content (RWC)

Relative water content was estimated as per the method of Barrs and Weatherly (1968) at 7,14 and 21 days after spraying of the pathogen/s. Twenty five leaf discs were collected from each plant and weighed accurately to three decimals on electronic balance. This was taken as fresh weight. The weighed leaf discs were floated in Petri dishes containing distilled water and allowed to take up water for four hours. After four h, leaf discs were blotted gently and weighed. This was referred to as the turgid weight. After taking turgid weight, the leaf discs were dried in an oven at 60-70°C for 48 h and dry weight was recorded. The RWC was calculated by using the following formula.

$$\text{RWC}(\%) = \frac{\text{Fresh weight - Dry Weight}}{\text{Turgid weight - Dry Weight}} \times 100$$

3.16.2.3 Dry matter accumulation

Leaf samples were collected from each treatment at 7, 14 and 21 days after spraying the pathogen/s. The dry weight of leaves was recorded and total dry matter accumulation was obtained.

3.16.2.4 Specific leaf weight

The specific leaf weight indicates the leaf thickness and this was determined by the method of Radford (1967).

$$\text{Specific leaf weight } (\text{mg/cm}^2) = \frac{\text{Leaf dry weight (mg)}}{\text{Leaf area } (\text{cm}^2)}$$

3.16.2.5 Estimation of Sugar by Anthrone method

Sugar content in leaves was estimated in oven dried samples at 7,14 and 21 days after spraying.

Dry sample extraction

1. Leaf dry powder (100 g) was taken in a conical flask and 10 ml of 80 per cent ethanol was added.
2. Contents were boiled on hot water bath for 10 minutes and contents were allowed to settle down. The supernatant was transferred to another dry flask.
3. To the residue in the flask, 10 ml of 80 per cent ethanol was added and extracted as before
4. Extraction was repeated again and the final volume was made up to 25 ml with 80 per cent ethanol.
5. From this, 5 ml of the extract was evaporated in a beaker on hot water bath (until the alcohol smell was lost) and made up the volume of the left out extract to 10 ml with distilled water. This was used for estimating sugar content as follows.

Standard curve

Glucose (100 mg) was dissolved in little quantity of water and the volume was made up to 100 ml to get a stock solution. From this, different concentrations were made from 10-100 $\mu g\ ml^{-1}$ by diluting and used for standard curve. The other procedure followed was similar to that used for plant samples.

Anthrone reagent

Anthrone (0.2 g) was dissolved in 100 ml of concentrated sulphuric acid. Fresh solution was prepared just before use.

Procedure

One ml of the aliquot having different concentrations (0,0.2,0.4,0.6,0.8 and 1.0 ml) of glucose standard solution were pipetted out in different test tubes. The volume was made up to 2.5 ml with distilled water. All the test tubes were kept in an ice bath to which 5 ml of anthorne reagent was added slowly. Contents were stirred gently with a glass rod and heated in a boiling water bath exactly for 7.5 min. and cooled immediately in an ice bath. After cooling the absorbence of the solution was measured at 630 nm against the blank in a spectrophotometer (Systronics model, CL-54) and the sugar content was calculated through the standard curve.

3.16.2.6 Estimation of tannins

Tannin content was estimated by Folin-Denis method in oven dried leaf samples at 7, 14 and 21 days after spraying (Schanderl, 1970).

Materials

1. Folin-Denis Reagent: One hundred gram of sodium tungstate and 20 g of phosphomolybdic acid were dissolved in 750 ml of distilled water. To which 50 ml of 85 per cent phosphoric acid was added. The mixture was reflexed for 2 h and the volume was made up to one litre with distilled water. The reagent was protected from exposure to light.

2. Sodium carbonate solution: Sodium carbonate (350 g) was dissolved in one litre of water at 70-80°C, filtered through glass wool after allowing it to stand overnight.

3. Standard Tannic acid solution: 100 mg of tannic acid was dissolved in 100 ml of distilled water (1 mg/1ml).

4. Working standard solution: Five ml of the stock solution was diluted to 100 ml with distilled water.

Procedure

Extraction of tannins: Powdered leaf material (0.5 g) was transferred to a 250 ml conical flask, to which 75 ml of water was added. The flask was heated gently and boiled for 30 minutes, centrifuged at 2000 rpm for 20 min. The supernatant was collected in volumetric flask and the volume was made up to 100 ml. From this one ml of the extract was transferred to a 100 ml volumetric flask containing 75 ml water, to which 5 ml of Folin-Penis reagent and 10 ml of sodium carbonate solution were added and the volume was made up to 100 ml with distilled water. The contents were shaken well and the absorbance was read at 700 nm after 30 minutes in a Spectrophotometer (Systronics model, CL-54). Blank was prepared with water instead of the sample. The absorbence was compared with standard graph by using 0-100 ug of tannic acid.

3.16.2.7 Estimation of Nitrate reductase activity

The nitrate reductase activity *in vivo* was assayed by the method of Sardhambal *et al.,* (1978) at 7,14 and 21 days after spraying of pathogens.

A stock solution, containing 0.1 M phosphate buffer (pH 7.5), propanol (5%) and 0.01 M KNO_3 was prepared. Leaf bits of known weight were suspended in 25 ml flask containing 5 ml of stock solution and two drops of Chloramphenicol (0.5 mg/ml). The flasks were incubated at 30°C for 20 minutes. After incubation, reaction was stopped by adding 0.1 ml zinc acetate and 1.9 ml ethanol (70%). The contents were mixed thoroughly and centrifuged at 3000 g for 10 min. and supernatant decanted. Nitrate formed was determined in aliquot of the supernatant by adding 1 ml of Sulphanilamide (1%) in 1M HI and 1 ml of N-1 naphthylethylene diamine dihydrochloride (0.02%). The absorbance was measured at 540 nm in a spectrophotometer (Systronics model CL-54) after 20 minutes. The activity of nitrate reductase was determined from a standard curve of $NaNO_2$ and expressed as μ mole NO_2 g fresh weight^{-1} min^{-1}.

3.16.2.8 Estimation of total Phenols

Estimation of total phenols present in the samples was carried out by Folin-Ciocalteau Reagent (FCR) method (Malick and Singh, 1980) in oven dried samples at 7, 14 and 21 days after spraying.

Reagents: 1) Folin - Ciocalteau Reagent (FCR) 1N

2) Sodium carbonate (2% $Na_2 Co_3$ in 0.1 N NaOH)

Procedure

One ml of the alcohol extract was taken in a test tube, to which one ml of Folin-Ciocalteau reagent followed by 2.0 ml of sodium carbonate solution were added. The tube were shaken well and heated in a boiling water bath for exactly one min. and then cooled under running tap water. The blue colour

developed was diluted to 25 ml with distilled water and its absorbance was read at 650 nm in a spectrophotometer (Systronics model, CL-54). The amount of phenols present in the sample was calculated from a standard curve prepared from catechol and expressed in mg per gram dry weight.

3.16.2.9 Estimation of total free Amino acids

The free amino acids were determined by adopting the procedure given by Moore and Stein (1948).

To a known quantity (1.0 ml) of alcohol extract one ml of ninhydrin reagent was added. The contents of the tubes were mixed thoroughly and kept for 20 min. in a boiling water bath. After cooling, volume was made up to 10 ml with 50 per cent n-propanol. The intensity of violet colour produced was read at 570 nm in a spectrophotometer. The quantity of free amino acids was calculated using glycine standard curve and expressed as mg per gram dry weight.

3.16.2.10 Biophysical studies

Measurements of various biophysical parameters *viz.*, photosynthetic rate, transpiration rate and leaf temperature were made on the adaxial surface of third fully expanded leaf from the top at 7,14 and 21 DAS after spraying of pathogens by using infra red gas analyser (Model, CI-301). The measurements were made between 9.30 am to 12.30 p.m. on all the sampling dates. The photosynthetic rate, transpiration rate and leaf temperature were expressed in terms of μ mol of CO_2 dm^{-2} sec^{-1}, μg H_2O m^{-2} S^{-1} and 0°C, respectively.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the investigation on management of eupatorium weed (*Chromolaena odorata*) through mycoherbicides are presented here under. The experiment were conducted in laboratory and glass house in Department of Plant Pathology, College of Agriculture, Dharwad during 1995-96 and field experiments were conducted at Farm Forestry Station, University of Agricultural Sciences, Dharwad during 1996-97.

4.1 Survey for diseases of *Chromolaena odorata*

A survey for the pathogens associated with *C. odorata* in endemic parts of Karnataka state viz., Belgaum, Dharwad, Uttar Kannada and Shimoga districts was undertaken during 1995-96. Survey was done at vegetative and reproductive weed growth stages as explained in 'Material and Methods'. The diseased samples were collected and symptoms on eupatorium weed were recorded.

4.1.1 Isolation of pathogens

The pathogens were isolated from the diseased parts of the weed by following standard tissue isolation technique. Subculturing of all pathogenic isolates was done once in 15 days on PDA. Isolated cultures were sent for identification to Agharkar Research Institute, Maharashtra Association for Cultivation Sciences, Pune (Maharashtra), India.

The results of the survey work and the pathogens associated with *C. odorata* are presented in the Table 1. From the perusal of the Table 1 and Fig.1, it is clear that, pathogens associated with eupatorium weed were all fungal species. Totally, eighteen isolates were isolated from different locations. During

Table 1: Fungal pathogens associated with *Chromolaena odorata* from Karnataka

	Location	Pathogens isolated	Plant part / parts affected
I.	**Belgaum**		
a.	Belgaum	*Alternaria alternata* (Fr.) Keissler	Leaf lamina
b.	Khanapur	*A. altemata* (Fr.) Keissler	Leaf lamina
c.	Londa	*Bipolaris* sp.	Leaf lamina
II.	**Dharwad**		
a.	Dharwad	a. *Colletotrichum gloeosporioides* (Penz.) Sacc.	Leaf lamina
	and	b. *Cladosporium sphaerospermum* Penz.	Leaf lamina
	Prabhunagar	c. *Colletotrichum gloeosporioides*	Leaf lamina
b.	Hubli	*A. altemata* (Fr.) Keissler	Leaf lamina
c.	Kalagatagi	*A. altemata* (Fr.) Keissler	Leaf lamina
III.	**Uttar Kannada**		
a.	Haliyal	*Phyllosticta* sp.	Leaf lamina
b.	Mundagod	a. *Phoma eupatorii* Died	Leaf lamina
		b. *Aureobasidium pullulans* (deBary) Arnaud	Influorescens
c.	Sirsi	a. *A. altemata* (Fr.) Keissler	Leaf lamina
		b. *Phomopsis* sp.	Leaf lamina Leaf lamina
		c. *Biopolaris* sp.	Leaf lamina
d.	Yellapur	*A. altemata* (Fr.) Keissler	Leaf lamina
IV.	**Shimoga**		
a.	Hosanagara	*A. altemata* (Fr.) Keissler	Leaf lamina
b.	Sagar	*Beltrania rhombica* Penz.	Leaf lamina
c.	Shimoga	*A. altemata* (Fr.) Keissler	Leaf lamina

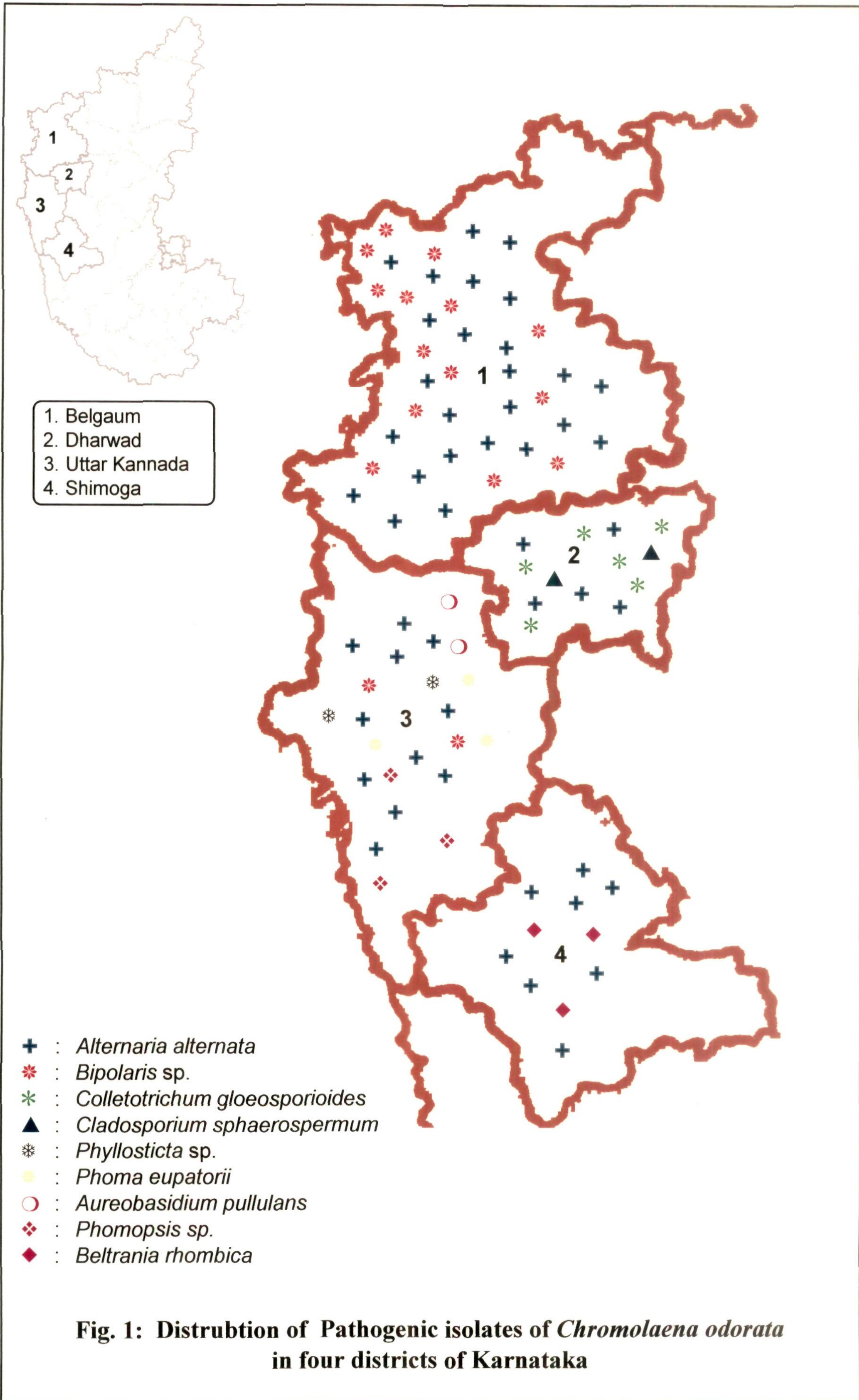


Fig. 1: Distrubtion of Pathogenic isolates of *Chromolaena odorata* in four districts of Karnataka

the survey, it was noticed that, *Alternata alternata* (Fr.) Keissler was found to be widely distributed and it was collected from eight localities viz., Belgaum, Shimoga, Hosanagar, Khanapur, Kalaghatagi, Sirsi, Yellapur and Hubli. It caused leaf spots and necrosis.

A floral pathogen *Aureobasidium pullulans* (deBary) Arnaud was collected from only one location i.e., Mundagod. *Beltrania rhombica* Penz. was recorded from only one location i.e. Sagar of Shimoga district. It caused leaf spots on eupatorium, whereas, *Bipolaris* sp. was isolated from two different talukas *viz.,* Sirsi and Londa. It affected leaf lamina and also caused leaf spots.

Cladosporium sphaerospermum Penz. was isolated from only one location, i.e. Dharwad. It was a foliar pathogen and caused few minute lesions on the foliage.

The other foliar pathogen isolated from Dharwad was *Colletotrichum gloeosporioides* (Penz.) Sacc. which was also isolated from diseased samples collected from Prabhunagar Forest areas of Dharwad district. The pathogens *Phoma eupatorii* Died, *Phomopsis* sp. and *Phyllosticta* sp. found to be associated with leaf lamina were isolated from Mundagod, Sirsi and Haliyal regions respectively.

Eighteen fungal isolates were isolated from eupatorium growing endemic areas of Karnataka which are distributed in nine different genera *viz., Alternaria, Aureobasidium, Beltronia, Bipolaris* sp., *Cladosporium, Colletotrichum, Phoma, Phomopsis* sp. and *Phyllosticta* sp. All organisms were foliar pathogens except *Aureobasidium pullulans* which was a floral pathogen.

4.1.2 Symptomatology

4.1.2.1 Symptoms caused by *Alternaria alternata*

(Deuteromycotina : Hyphomycetes : Moniliales : Dematiaceae)

The fungus caused leaf spots, which were dark brown with paler margin surround by a yellow halo. The spots were first smaller in size, then gradually increased upto 1-2 cm, with concentric rings or target board symptoms with irregular or circular shape. Later, the lesions became very brittle, leading to premature defoliation. During survey, it was observed that, samples collected from Yellapur taluk showed veinal necrosis and drooping of young branches.

4.1.2.2. Symptoms caused by *Aureobasidium pullulans* (=*Pullularia pullulans*)

(Deuteromycotina : Hyphomycetes : Moniliales : Dematiaceae)

Earlier, the pathogen was included under yeasts and called as black yeasts. In the present investigation, the pathogen was found to be associated with flowers of eupatorium. It resulted in black mouldy growth covering the entire flower buds and flowers. However, few mild lesions were produced on leaves. Severe infection on flower buds resulted in premature dropping of buds and production of malformed or sterile seeds.

4.2.2.3 Symptoms caused by *Beltrania rhombica*

(Deuteromycotina : Hyphomycetes : Moniliales : Dematiaceae)

The pathogen caused small chlorotic lesions on the older leaves, which turned necrotic with white center. Individual lesions measured 1-2 mm in diameter. Rarely the spots coalesced giving a blighted appearance.

4.1.2.4 Symptoms caused by *Bipolaris* sp.

(Deuteromycotina : Hyphomycetes : Moniliales : Dematiaceae)

The fungus caused leaf spots, which were brown, round to oval, measuring about 1-5 mm. They were usually isolated but in severe cases coalesced to form large patches.

4.1.2.5 Symptoms caused by *Cladosporium sphaerospermum* Penz.

(Deuteromycotina : Hyphomycetes : Moniliales : Dematiaceae)

The first symptom of the disease was the development of chlorotic patches on the upper surface of the leaves. On the corresponding lower surface, brown discoloured spots were noticed.

4.1.2.6 Symptoms caused by *Colletotrichum gloeosporioides*

(Deuteromycotina : Coelomycetes : Melanconiales: Melanconiceae)

The pathogen caused leaf spots on eupatorium. The spots were very minute at first in the centre or on the margin, which later enlarged and coalesced to form larger spots. Later, such coalesced spots became brittle and the infected part was amputated, resulting in `shot hole' symptoms on the leaves. Defoliation was also noticed. The spots were mostly irregular, rarely circular, brownish to black in colour. It was noticed that, *C. gloeosporioides* infected more on younger leaves.

4.1.2.7 Symptoms caused by *Phoma eupatorii* (Deuteromycotina : Coelomycetes : Sphaeropsidales : Sphaeropsidaceae)

The pathogen caused leaf spots. In the beginning, small round to irregular spots appeared with light brown centre. Later spots increased in size. The central portions of these spots turned brown, later dried into white membranous patch. In older spots, black dot like erumpent scattered fruiting bodies were rarely observed.

4.1.2.8 Symptoms caused by *Phomopsis* sp. (Deuteromycotina : Coelomycetes : Sphaeropsidales : Sphaeropsidaceae)

The pathogen caused leaf spots. Spots were few to many, minute, bold, scattered all over the leaf. Spots were circular to irregular in shape with light brown centre surrounded by dark brown margin.

4.1.2.9 *Phyllosticta* sp. (Deuteromycotina : Coelomycetes : Sphaeropsidales : Sphaeropsidaceae)

Lesions were small, 0.5-1.0 cm. Circular to irregular, reddish brown on upper surface of the leaf. These spots rarely coalesced each other covering larger areas of the leaf.

4.1.3 Isolation of pathogens and proving the pathogenicity

All the pathogenic isolates of eupatorium were successfully isolated from the infected leaves / flower and pure culture of each isolate was obtained. Artificial inoculation on the weed plant was carried out as described in 'Material

and Methods'. It was noticed that all fungal isolates were pathogenic to eupatorium but at varying degree i.e few minute lesions to severe necrosis. The typical symptoms were produced on the leaves after seventh day of inoculation. *A. pullulans* produced black mould of flowers after seven days of inoculation. Plants in the control pot remained healthy.

4.1.4 Screening and selection of virulent pathogens of *Chromolaena odorata*

This experiment was conducted to select only the potential isolates among eighteen pathogenic isolates. Selection was made by assessing the aggressiveness of all the isolates to induce disease severity on tenth day and its relative increase in PDI on fifteenth day after spray inoculation on eupatorium.

The experiment was carriedout under glass house conditions as explained in 'Material and Methods'. The results (Table 2) revealed that, all the pathogenic isolates had capacity to produce symptoms at varying degrees.

Among the eight isolates of *Alternaria alternata*, Yellapur isolate was found to be highly virulent over other isolates of *A. alternata*. Yellapur isolate showed significantly higher PDI (62.50%) on tenth day, which drastically increased on fifteenth day (80.00%) when compared to other isolates of *A. alternata*. In addition to typical target board symptoms, veinal necrosis was also noticed only in plants inoculated with Yellapur isolate. Hence, among the eight isolates of *A. alternata*, Yellapur isolate was selected, as it was more virulent.

Virulence of two isolates of *Colletotrichum gloeosporioides* (Dharwad and Prabhunagar isolates) was significantly on par with each other, with 87.00 and 92.00 per cent disease index on fifteenth day respectively. Hence, both the

Table 2: Virulence of different pathogenic isolates infecting *Chromolaena odorata*

Sl.No.	Pathogens	Isolates	Per cent disease index	
			10 days	15 days
1.	*Alternaria alternata*	Sirsi	44.00 (41.55)*	66.50 (52.24)
2.	*A. alternata*	Yellapur	62.50 (52.24)	80.00 (63.44)
3.	*A. alternata*	Hubli	49.00 (44.43)	59.00 (50.18)
4.	*A. alternata*	Mundagod	42.50 (40.69)	50.00 (45.00)
5.	*A. alternata*	Shimoga	39.00 (38.65)	45.00 (42.13)
6.	*A. alternata*	Hosanagara	39.00 (38.65)	49.00 (44.43)
7.	*A. alternata*	Belgaum	41.00 (39.82)	42.00 (40.40)
8.	*A. alternata*	Kalaghatagi	33.00 (35.06)	35.50 (36.57)
9.	*Aureobasidium pullulans*	Mundagod	85.00 (67.21)	100.00 (90.00)
10.	*Bipolaris* sp.	Sirsi	28.00 (31.95)	28.50 (32.27)
11.	*Bipolaris* sp.	Londa	22.00 (27.97)	22.00 (27.97)
12.	*Beltrania rhombica*	Sagar	19.00 (25.84)	20.00 (26.56)
13.	*Cladosporium spherospermum*	Dharwad	26.50 (30.98)	28.00 (31.95)
14.	*Colletotrichum gloeosporioides*	Dharwad	72.50 (58.37)	87.00 (68.87)
15.	*C. gloeosporioides*	Prabhunagar	75.00 (60.00)	92.00 (73.57)
16.	*Phyllosticta* sp.	Haliyal	37.50 (37.76)	52.00 (46.15)
17.	*Phoma eupatorii*	Mundagod	35.00 (36.27)	59.00 (50.18)
18.	*Phomopsis* sp.	Sirsi	42.00 (40.40)	58.00 (49.60)
	S. Em±		1.83	1.52
	CD at 1%		7.56	6.28

*Figures in parentheses indicate arc sine transformed value

isolates were selected for further screening. However, the isolates were significantly superior over *A. alternata* with respect to virulence.

Phoma eupatorii, Phomopsis sp. and *Phyllosticta* sp. were found to be moderately virulent with PDI 59.00, 58.00 and 52.00, respectively on 15th day and they were included in the second step-screening programme.

Beltrania rhombica, Cladosporium herbarum and two isolates of *Bipolaris* sp. were found to be less virulent with significantly low PDI on fifteenth day after spraying (20.00%, 20.00% (Londa), 28.50% (Sirsi) and 28.00%, respectively). Disease level was not increased even after fifteenth day of incubation. Due to their low virulence and inability to enhance disease pressure on eupatorium they were deleted from the study.

Aureobasidium pullulans caused cent per cent black mould of flowers and it was found to be very potential pathogen.

From the results, it can be concluded that, *C. gloeosporioides* (Dharwad and Prabhunagar isolates), *A. alternata* (Yellapur isolate), *Phoma eupatorii, Phomopsis* sp. and *Phyllosticta* sp. as foliar pathogens and *Aureobasidium pullulans* (Mundagod) as a floral pathogen can be included in the second step screening to select highly virulent and potential pathogens in the management of eupatorium weed.

4.2 Cultural and morphological characters of pathogens of *Chromolaena odorata*

A study on cultural and morphological characteristics of pathogens of *C. odorata* was carried out on nine different solid media which included synthetic, and non synthetic media. The experiment was conducted under laboratory

conditions at a room temperature of 27 ± 1 °C as explained in 'Material and Methods'.

The objective of this experiment was to screen and select only the potential virulent pathogens of eupatorium among the seven pathogenic isolates based on their capacity to grow and sporulate on media. The medium, that supported best growth and excellent sporulation, was selected as 'basal medium' for mass multiplication, physiological, nutritional and biochemical, studies of potential pathogens. The different parameters considered in this experiments were viz., colony characters, colony diameter, colour of the colony, type of colony and sporulation.

4.2.1 Growth characteristics on different solid media

Variation in colony characters viz., colour of the colony, type of growth and colony margin among the seven pathogenic isolates of eupatorium are presented (Table 3 & 4, Fig. 2 & 3 and Plates 3-6)

4.2.1.1 Non-synthetic media

Host leaf extract agar

All pathogenic isolates were light black to dark black, with submerged growth and smooth colony margin except isolate D (*Phoma eupatorii*) and E (*Phyllosticta* sp.) which showed light coloured colony and elevated growth respectively.

Host leaf extract agar + 1% sucrose

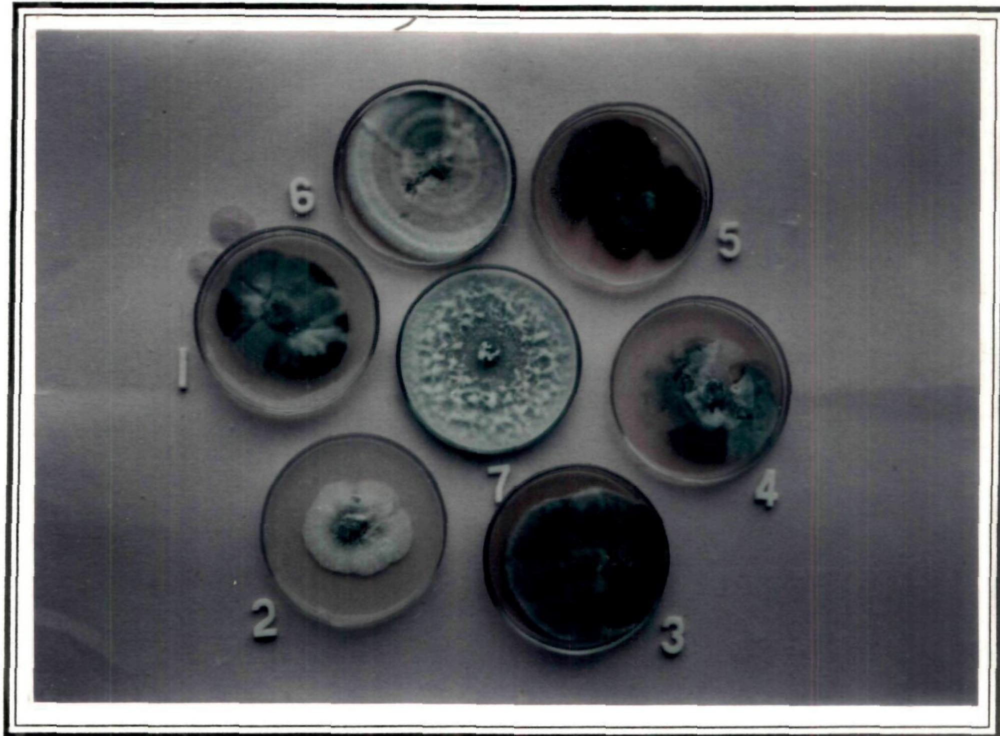
Colour of isolates was usually light black to dark black with submerged growth and smooth colony margin. Whereas G (*Aureobasidium pullulans*) and

Table 3. Colony characteristics of seven pathogenic isolates of *Chromolaena odorata* on different non-synthetic media

Sl.No.	Non-synthetic media	Isolates			
		A	B	C	D
1.	Host extract + 1% sucrose agar medium	Light black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Light black coloured colony, Submerged growth, Smooth colony margin	Light red coloured colony. Submerged growth, Smooth colony margin
2.	Host extract agar medium	Light black colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Light black coloured colony, Submerged growth, Smooth colony margin	Light red coloured colony, Submerged growth, Smooth colony margin
3.	Oat meal agar medium	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Light black coloured colony, Submerged growth, Smooth colony margin	Light red coloured colony, Submerged growth, Smooth colony margin
4.	Potato dextrose agar medium	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Red coloured colony, Submerged growth, Smooth colony margin

Contd…

Sl.No.	Non-synthetic media	Isolates		
		E	F	G
1.	Host extract + 1% sucrose agar medium	Black coloured colony, Elevated growth, Smooth colony margin	Black coloured colony, Smooth colony margin, Submerged growth	Black coloured colony, Smooth colony margin, Elevated growth
2.	Host extract agar medium	Black coloured colony, Elevated growth, Smooth colony margin	Black coloured colony, Smooth colony margin, Submerged growth	Light black coloured colony, Smooth colony margin, submerged growth
3.	Oat meal agar medium	Black coloured colony, Elevated growth, Serrated colony margin	Black coloured colony, Smooth colony margin, Elevated growth	Light black coloured colony, Smooth colony margin, Submerged growth
4.	Potato dextrose agar medium	Black coloured colony, Elevated growth, Serrated colony margin	Black coloured colony, Elevated growth, Serrated colony margin	Black coloured colony, Elevated growth, Serrated colony margin



1. A. alternata, 2. *Phomopsis sp.,* 3. *C. gloeosporioides* (Prabhunagar),
4. *C. gloeosporioides* (Dharwad), 5. *Phyllostica* sp., 6. *Phoma eupatorii,* 7. *A. pullulans*

Plate 3. Growth of different isolates on potato dextrose agar medium

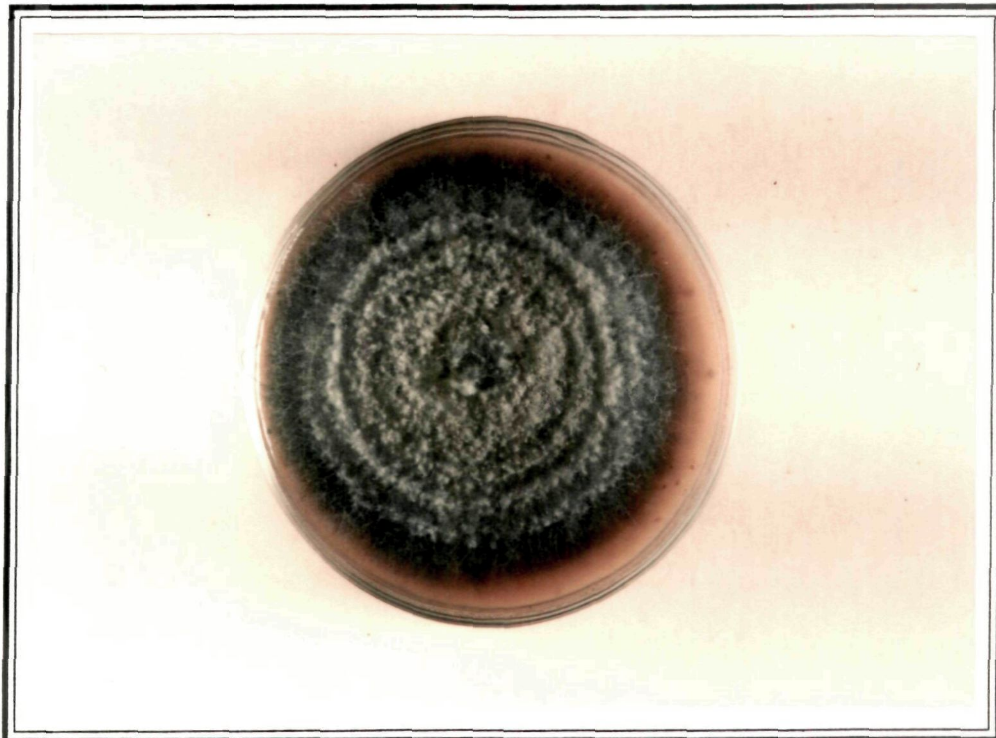


Plate 4. Concentric ring growth pattern of *Aureobasidium pullulans*

E (*Phyllosticta* sp). showed elevated growth, while D (*Phoma eupatorii*) had red coloured colony.

Oat meal agar

Isolates A (*Colletotrichum gloeosporioides,* Prabhunagar), B *Colletotrichum gloeosporioides*, Dharwad), C (*A. alternata*, Yellapur) and G (*Aureobasidium pullulans*) showed light black to black coloured colony, submerged growth and smooth colony margin. Whereas, isolate D showed red colony and elevated growth was observed in isolates E and F.

Potato dextrose agar

Colour of all the isolates was black except D, which was red. Colony margin was smooth in all the isolates, however, E isolate showed serrated margin. Isolates E, F and G showed elevated growth whereas, growth was submerged in other isolates.

Synthetic media

Czapek's agar

Isolates A, B, C, F and G were light to dark black with submerged growth and smooth colony margin whereas, isolate D and E exhibited red colony and *elevated growth with serrated colony margin respectively.*

Czapek's dox agar

Colony characters were same as in Czapek's agar medium.

Richards's agar

Colony characters were same as in that of Czapek's agar medium.

Table 4. Colony characteristics of seven pathogenic isolates of *Chromolaena odorata* on different synthetic media

Sl.No.	Synthetic media	Isolates			
		A	B	C	D
1.	Czapek's agar medium	Black colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth colony margin	Black colony, Submerged growth, Smooth margin	Red coloured colony, Submerged growth, Smooth margin
2.	Czapek's Dox agar medium	Black colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Red coloured colony, Elevated growth, Smooth margin
3.	Richards's agar medium	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Red coloured colony, Submerged growth, Smooth colony margin
4.	Sabouraud's Dextrose agar medium	Black colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Red coloured colony, Elevated growth, Smooth margin
5.	Tochinai's agar medium	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Black coloured colony, Submerged growth, Smooth colony margin	Red coloured colony, Slightly elevated growth, Smooth margin

Contd...

Sl.No.	Synthetic media	Isolates		
		E	F	G
1.	Czapek's agar medium	Light black coloured colony, Elevated growth, Serrated margin	Light black coloured colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth margin
2.	Czapek's Dox agar medium	Light black coloured colony, Elevated growth, Serrated margin	Light black coloured colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth margin
3.	Richards's agar medium	Light black coloured colony, Elevated growth, Serrated margin	Light black coloured colony, Smooth margin, Submerged growth	Black coloured colony, Submerged growth, Smooth margin
4.	Sabouraud's Dextrose agar medium	Light black coloured colony, Elevated growth, Serrated margin	Light black coloured colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth margin
5.	Tochinai's agar medium	Light black coloured colony, Elevated growth, Serrated margin	Light black coloured colony, Submerged growth, Smooth margin	Black coloured colony, Submerged growth, Smooth margin

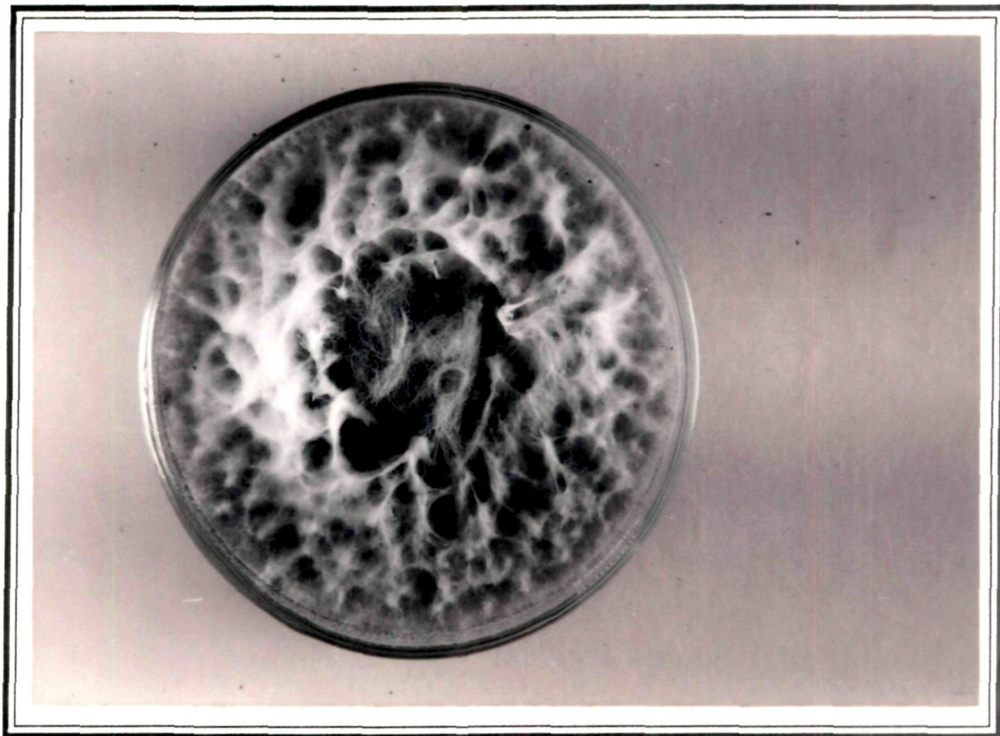


Plate 5. Typical aerial mycelial growth of *Phyllosticta* sp.

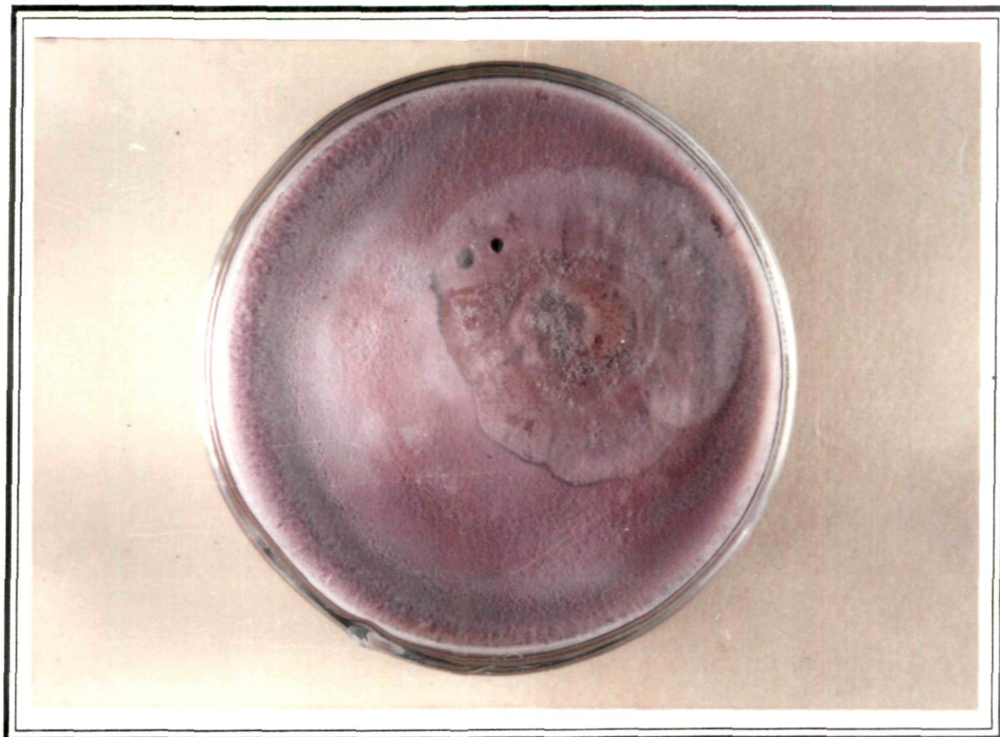


Plate 6. Colony character of *Phoma eupatorii* on potato dextrose agar medium

Sabouraud's dextrose agar

Colonies were light black to dark black coloured with submerged growth and smooth margin in isolates A, B, C, F and G. However, isolate D and E differed from all the above isolates only with respect to red coloured colony and elevated growth with serrated colony margin respectively.

Tochinai's agar

All the isolates were light to dark black coloured with submerged growth and smooth colony margin except E, G and D which were of serrated margin and red coloured colony, respectively.

4.2.2 Colony diameter and sporulation of pathogenic isolates on different solid media

The results are presented in the Table 5 and 6 revealed significant differences between isolates, media and also interaction.

Maximum radial growth (61.67 mm) was recorded in pathogenic isolate G which was significantly on par with pathogen D (60.55 mm). However, excellent sporulation (3.84) was noticed in isolate G, where as isolate D sporulated rarely (0.11).

Next best was the pathogenic isolate A, with respect to its radial growth (58.33 mm) and excellent sporulation (3.50). Though isolate F exhibited same growth rate (58.33 mm), but sporulated sparsely (0.33). Even though, isolate C recorded least radial growth (35.78 mm) but it was able to sporulate heavily (2.22).

Table 5. **Colony diameter and sporulation capacity of seven pathogenic isolates of *Chromolaena odorata* on different synthetic and non-synthetic media**

Sl. No.	Media	Isolates							
		A		B		C		D	
		Colony diameter (mm)	Sporulation	Colony diameter (mm)	Sporulation	Colony diameter (mm)	Sporulation	Colony diameter (mm)	Sporulation
	Non-synthetic media								
1	Host extract + 1% sucrose agar	65	Excellent	50	Good	35	Good	45	Nil
2	Host extract agar	65	Excellent	60	Excellent	35	Fair	45	Nil
3	Oat meal agar	60	Fair	55	Poor	40	Poor	55	Nil
4	Potato dextrose agar	80	Excellent	75	Good	40	Excellent	40	Poor
	Synthetic media								
1	Czapek's agar	50	Excellent	53	Poor	35	Fair	70	Nil
2	Czapeks's Dox'sagar	50	Good	50	Fair	32	Fair	75	Nil
3	Richards's agar	50	Excellent	40	Excellent	40	Good	80	Poor
4	Sabouraud's dextrose agar	75	Excellent	66	Excellent	40	Poor	85	Nil
5	Tochinai's agar	25	Fair	20	Good	25	Good	50	Nil
	Mean	58.33		50.88		35.78		60.55	

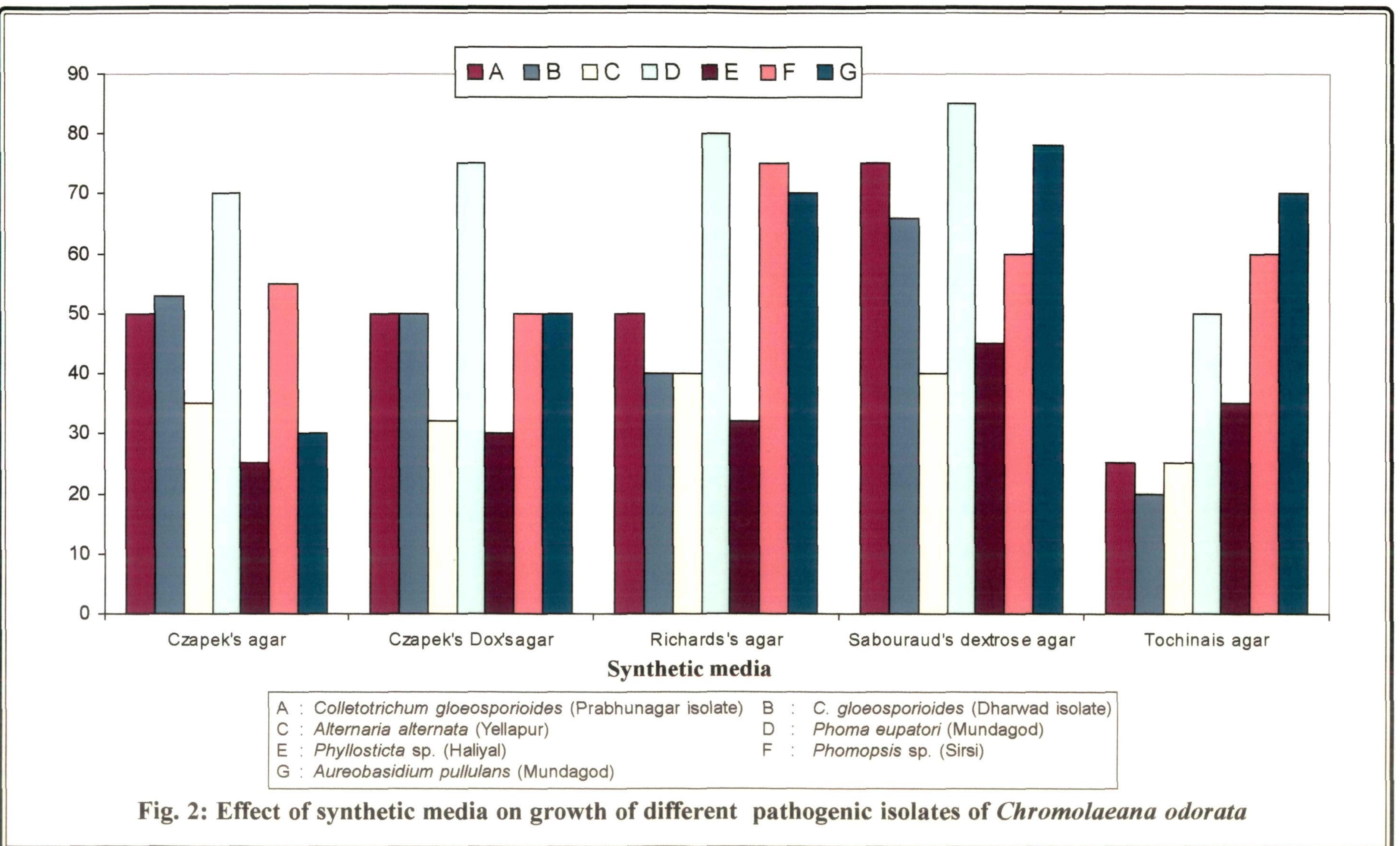


Fig. 2: Effect of synthetic media on growth of different pathogenic isolates of *Chromolaeana odorata*

Table 5. Contd...

Sl. No.	Media	Isolates						Mean
		E		F		G		
		Colony diameter (mm)	Sporulation	Colony diameter (mm)	Sporulation	Colony diameter (mm)	Sporulation	
	Non-synthetic media							
1	Host extract + 1% sucrose agar	45	Nil	40	Poor	55	Excellent	37.22
2	Host extract agar	35	Nil	40	Poor	40	Excellent	35.55
3	Oat meal agar	55	Nil	60	Nil	75	Excellent	43.33
4	Potato dextrose agar	90	Poor	85	Poor	85	Excellent	55.00
	Synthetic media							
5	Czapek's agar	25	Nil	55	Nil	30	Excellent	35.33
6	Czapek's Dox'sagar	30	Nil	50	Nil	50	Excellent	37.44
7	Richard's agar	32	Nil	75	Nil	70	Excellent	44.66
8	Sabouraud's dextrose agar	45	Nil	60	Nil	78	Excellent	48.88
9	Tochinai's agar	35	Nil	60	Nil	70	Good	31.67
	Mean	43.56		58.33		61.67		

For comparing	S.Em±	CD at 1%
Isolate	0.07	0.19
Media	0.08	0.22
Isolate x Media	0.21	0.59

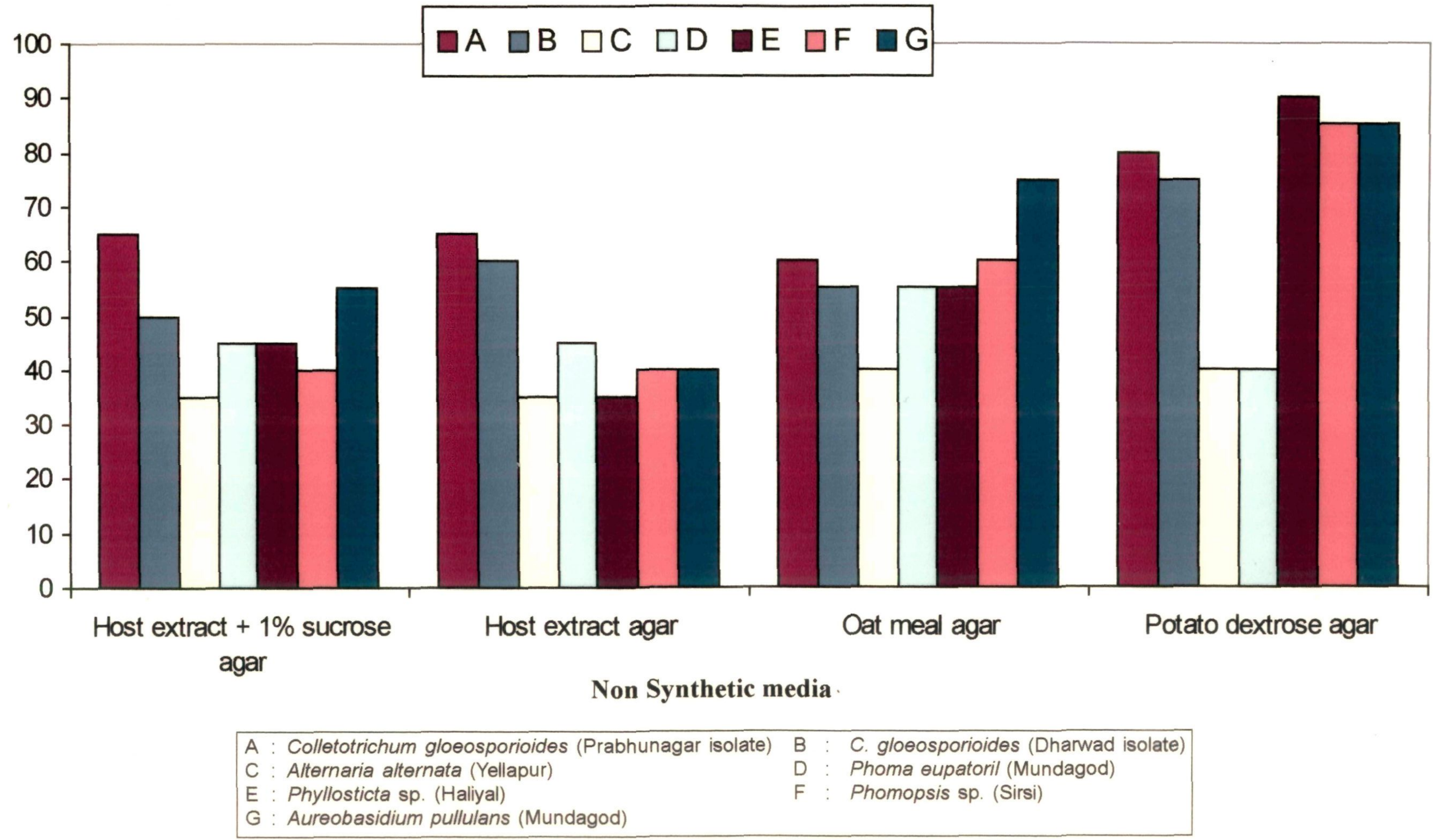


Fig. 3: Effect of non synthetic media on growth of different pathogenic isolates of *Chromolaeana odorata*

Table 6. Relative amount of sporulation of pathogenic isolates of *Chromolaena odorata* on different solid media

Pathogenic isolates	Sporulation on media									
	Host extract agar	Host extract agar + 1% sucrose	Oat meal agar	PDA	Sabourad's dextrose agar	Czapek's agar	Czapek's don's agar	Richard's agar	Tochinais agar	Mean
A	4	4	2	4	4	4	3	4	2	3.50
B	4	4	1	3	4	1	1	3	3	2.66
C	2	3	1	4	1	2	2	3	3	2.22
D	0	0	0	1	0	0	0	1	0	0.22
E	0	0	0	1	0	0	0	0	0	0.11
F	1	1	0	1	0	0	0	0	0	0.33
G	4	4	3	4	4	4	4	4	4	3.84
Mean	2.14	2.28	1.0	2.57	1.75	1.57	1.57	2.14	1.71	-

A : *Colletotrichum gloeosporioides* (Prabhunagar isolate)
B : *C. gloeosporioides* (Dharwad isolate)
C : *Alternaria alternata* (Yellapur)
D : *Phoma eupatorii* (Mundagod)
E : *Phyllosticta* sp. (Haliyal)
F : *Phomopsis* sp. (Sirsi)
G : *Aureobasidium pullulans* (Mundagod)

Sporulation	No. of spores / microscopic field	Grade
Excellent	> 70	4
Good	50-70	3
Fair	20-50	2
Poor	<20	1
Nil	0	0

Among the media, potato dextrose agar supported maximum radial growth (55.00 mm) followed by Sabouraud's dextrose agar (48.88 mm). Among the synthetic media, Richards's agar supported highest growth with mean radial growth of 44.66 mm). Tochinai's agar yielded minimum mean mycelial growth (31.67 mm).

Maximum sporulation was noticed in potato dextrose agar (2.57) followed by Host extract agar + 1% sucrose (2.28), host extract agar (2.14) and Richards's agar (2.14).

Significant interaction indicated the variation in virulence of pathogenic isolates in utilizing the media. Radial growth of isolates E (90.00 mm); F (85.00 mm) and G (85.00 mm) were significant to remaining ones on potato dextrose agar. Other media also showed variation in virulence on other pathogenic isolates.

From the results, it is evident that, pathogenic isolates G (*Aureobasidium pullulans*) and A (*Colletotrichum gloeosporioides,* Prabhunagar) were the most potential in respect to mycelial growth and sporulation. *C. gloeosporioides* of Prabhunagar isolate sporulated more efficiently than Dharwad isolate. Therefore Prabhunagar isolate was selected for further studies. Isolate C (*Alternaria alternata*, Yellapur) was a good sporulator with least mycelial growth. Though, isolates D, E, F showed profused mycelial growth, sporulation was negligible. Based on these results, isolates A, C and G were selected as 'potential virulent pathogens and they were used in further studies.

4.2.3 Growth phase

This experiment was conducted to ascertain the maximum growth period of all the three potential pathogens in potato dextrose broth. The growth in

inoculated flasks was harvested at two days interval from 4th day onwards upto 20th day after inoculation and the dry mycelial weight recorded is given in Table 7.

Dry mycelial weight of *Alternaria alternata* was minimum on 4th day of seeding (121.55 mg) was significantly increased upto 12th day of incubation. Growth was maximum (328.66 mg) on 12th day of seeding and latter it decreased substantially. Hence, 12th day of seeding was considered as the optimum period for further physiological and nutritional studies of *A. alternata*

Mycelial growth of both the pathogens, *C. gloeosporioides* and *A. pullulans* reached peak on 10th day of seeding (297.63 mg and 323.00 mg, respectively) and significantly more than other periods. Growth of these two pathogens was minimum at the incubation period of four days with 98.00 mg and 165.00 mg, respectively.

Since maximum dry mycelial weight was observed on the 10th day of seeding of *C. gloeosporioides* and *A. pullulans*, hence, it was considered as the optimum period for further physiological and nutritional studies.

4.2.4 Growth studies in liquid media

This experiment was conducted to know the best medium for the growth of each pathogen on dry mycelial weight basis. Mycelial growth was harvested on 10th day and 12th day of seeding of *C. gloeosporioides, A. pullulans* and *A. alternata*, respectively.

The results of this study are presented in the Table 8.The results revealed that, maximum growth of *A. alternata* was observed in potato dextrose broth (330.43 mg) as well as in Richards's media (330.40 mg). This was significantly

Table 7: Dry mycelial weight of potential pathogens of *Chromolaena odorata* in potato dextrose broth at two days interval from 4th to 20th day after inoculation

Sl. No.	Days after inoculation	Mean dry mycelial weight (mg)		
		A	B	C
1	4	121.55	98.00	165.00
2	6	195.00	163.00	220.25
3	8	255.02	204.15	301.00
4	10	301.89	297.63	323.00
5	12	328.66	257.00	273.35
6	14	310.00	220.22	234.66
7	16	268.18	175.14	185.92
8	18	235.43	108.92	132.49
9	20	185.75	79.96	95.53
	S.Em±	1.63	1.63	1.91
	CD at 1%	6.72	6.72	7.91

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

Table 8: Dry mycelial weight of potential pathogens of *Chromolaena odorata* in different liquid media

Sl. No.	Liquid medium	Mean dry mycelial weight (mg)		
		A	B	C
	Non synthetic media			
1.	Host extract broth	250.79	265.66	203.83
2.	Host extract + 1% Sucrose	259.37	268.52	218.40
3.	Oat meal medium	328.00	248.67	271.00
4.	Potato dextrose broth	330.43	301.10	311.94
	Synthetic media			
5.	Czapek's medium	262.20	260.88	95.00
6.	Czapek's Doxs medium	213.58	238.39	158.44
7.	Richards's medium	330.40	268.00	265.17
8.	Sabouraud's dextrose medium	328.88	284.00	280.35
9.	Tochinai's medium	215.57	103.73	265.20
	SEm±	0.87	0.87	1.72
	CD at 1%	3.58	3.60	7.10

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

on par with Sabouraud's dextrose broth (328.88 mg) and out meal broth (328.00 mg). Least growth was recorded in Czapek's dox's medium (213.58 mg) followed by Tochinai's medium (215.57 mg), host extract broth (250.79 mg) and host extract +1% sucrose broth (259.37 mg).

Mycelial growth of *C. gloeosporioides* was maximum in potato dextrose broth (301.10 mg) which was significantly superior over other media tested. Next best was Sabouraud's dextrose broth (284.00 mg) followed by host extract + 1% sucrose (268.52 mg) and Richards's medium (268.00 mg). All other treatments were inferior to above mentioned treatments and growth was least in Tochinai's medium (103.73 mg).

Mean dry mycelial weight of *A. pullulans* was maximum in potato dextrose broth (311.94 mg) followed by Sabouraud's dextrose broth (280.35 mg). However, all the treatments differed significantly with respect to growth, except Richards's medium (265.17 mg) and Tochinai's medium (265.20 mg). Least growth was recorded in Czapek's medium (95.00 mg). Hence, further physiological and nutritional studies were conducted in Richards's medium wherever, a synthetic medium was required otherwise, Sabouraud's dextrose broth was used in the experiments.

4.3 Physiological studies of potential pathogens

4.3.1 Temperature requirement

This experiment was conducted to know the optimum temperature requirement for the maximum growth of the pathogens of Eupatorium. Different temperature levels i.e. 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, and 45°C were tested as explained in the 'Material and Methods' and the results are presented in the Table 9.

Table 9. Dry mycelial weight of potential pathogens of *Chromolaena odorata* at different temperature levels

Sl.No.	Temperature (°C)	Mean dry mycelial weight (mg)		
		A	B	C
1	0	0.00	0.00	0.00
2	5	0.00	0.00	0.00
3	10	13.00	24.67	0.00
4	15	64.67	74.33	78.67
5	20	178.67	180.67	203.86
6	25	300.00	290.33	272.00
7	30	279.83	294.67	281.00
8	35	112.33	75.67	226.13
9	40	59.00	49.33	75.00
10	45	0.00	0.00	0.00
	S.Em.±	1.48	1.17	3.09
	CD at 1%	6.43	5.05	12.78

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

Alternaria alternata produced maximum mycelial growth at 25°C (300.00 mg) which was significantly superior over other treatments. Next best was 30°C with dry mycelial weight of 279.33 mg. The pathogen failed to grow at 0°C, 5°C and 45°C. Growth was least at 10°C (13.00 mg) followed by 40°C (59.00 mg), 15°C (64.67 mg), 35°C (112.33 mg) and 20°C (178.67 mg).

Mycelial growth of *C. gloeosporioides* was minimum at 10°C (24.67mg) which gradually increased along with increase in temperature and reached peak at 30°C (294.67 mg). Significantly on par result was recorded at 25°C (290.33 mg). Growth at other temperatures was found to be significantly inferior. Growth was completely ceased at 0°C, 5°C and 45°C.

Aureobasidium pullulans failed to grow at 0°C, 5°C, 10°C, 40°C and 45°C. Poor growth was recorded at 40°C (75.00 mg) and 15°C (78.67 mg). Maximum growth was recorded at 30°C (280.00 mg) which was significantly on par with 25°C (272.00 mg). Good growth was noticed even at 35°C (226.13 mg) and 20°C (203.86 mg). However, it failed to sustain extreme temperatures.

From the results, it is clear that 25°C was optimum temperature for the growth of *A. alternata* and 30°C was best for the growth of *C. gloeosporioides* and *A. pullulans.*

4.3.2 Effect of hydrogen ion concentration on pathogens

This experiment was conducted to know the effect of pH levels on the growth of potential pathogens of eupatorium. The results are presented in Table 10.

Alternaria alternata failed to grow below pH 5.0 and above pH 7.50. Maximum mycelial growth was recorded at pH 5.50 (212.33 mg) and

Table 10. Dry mycelial weight of potential pathogens of *Chromolaena odorata* at different pH levels

pH levels	Mean dry mycelial weight (mg)		
	A.	B	C
4.00	0.00	0.00	109.72
4.50	0.00	0.00	125.18
5.00	157.00	98.76	140.95
5.50	212.33	132.36	260.00
6.00	176.14	191.00	283.00
6.50	121.92	223.13	258.34
7.00	103.68	135.88	240.17
7.50	45.11	93.91	260.13
8.00	0.00	0.00	281.61
8.50	0.00	0.00	26.00
S.Em±	0.67	0.57	1.03
CD at 1%	2.75	2.36	4.25

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

decreased significantly at other pH levels. Least growth was at pH 7.50 (45.11 mg). All the treatments differed significantly among themselves with respect to mycelial growth. Dry mycelial weight recorded at pH 5.00, 6.00, 6.50 and 7.00 was 157.00 mg, 176.14 mg, 121.92mg, 121.97 mg and 103.68 mg, respectively.

Colletotrichum gloeosporioides could not grow at pH 4.00, 4.50, 8.00 and 8.50. Growth was significantly increased from pH 5.00 (98.76 mg) and reached maximum at pH 6.50 (223.13 mg). Further, the growth started declining at pH 7.00 (135.88 no.) and 7.50 (93.91 mg). However, mycelial growth differed significantly in all the treatments.

In case of *A. pullulans* growth pattern was entirely different. It could grow well on a wide range of pH 4.0 to pH 8.0. Mycelial growth was good to moderate at all pH levels. However, maximum growth was recorded at pH 6.0(283.00 mg) and pH 8.00 (281.61 mg) which were significantly on par. Next best growth was at pH 5.50 (260.00 mg), 7.50 (260.13 mg) and 6.50 (258.34 mg) which were significantly on par with each other. At pH 8.50 growth was checked (26.00 mg).

From the results, it is clear that maximum growth of *A. alternata* and *C. gloeosporioides* was recorded at pH 5.50 and 6.50, respectively. Maximum growth of *A. pullulans* was favoured at pH 6.0 and 8.0.

4.4 Nutritional and biochemical studies

4.4.1 Effect of different carbon sources on the growth of potential pathogens

Utilization of seven different carbon sources by potential pathogens was studied as explained in 'Material and Methods' and the results are presented in the Table 11.

Table 11. Dry mycelial weight of potential pathogens of *Chromolaena odorata* in different carbon sources

Sl. No.	Carbon source	Mean dry mycelial weight (mg)		
		A	B	C
1	Citric acid	40.69	133.78	78.68
2	Dextrose	328.65	288.99	300.55
3	Fructose	233.03	179.59	248.00
4	Glucose	254.78	215.67	281.46
5	Lactose	179.00	105.33	230.46
6	Mannitol	210.33	200.00	248.98
7	Sucrose	325.00	261.00	278.12
	S.Em±	1.65	0.93	1.25
	CD at 1%	7.14	4.00	5.39

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

Maximum growth of *A. alternata* was recovered when dextrose was used as carbon source (328.65 mg), significantly followed by sucrose (325.00 mg) which were significantly superior to all other carbon sources tested. Next best carbon sources was glucose (254.78 mg) followed by fructose (233.03 mg) and mannitol (210.33 mg). Lactose (179.00 mg) and citric acid (40.69 mg) were least preferred by *A. alternata*.

C. gloeosporioides showed maximum growth in dextrose (288.99) which was significantly superior over other carbon sucrose tested. Mycelial growth differed significantly in all the carbon sources studied. Next best carbon source was sucrose (261.00 mg) followed by glucose (215.67 mg) mannitol (200.00 mg), fructose (179.59 mg) and citric acid (133.78 mg). Least growth was recorded in lactose (105.33 mg).

Highest growth of *A. pullulans* was recorded when dextrose was used as carbon source (300.00 mg). Next best growth was recorded in glucose (281.00 mg) which was significantly on par with sucrose (278.12 mg). Least growth (78.68 mg) was recorded in citric acid.

From the results, it is clear that dextrose was the best carbon source for the growth of all the three potential pathogens.

4.4.2 Effect of different nitrogen sources on the growth of potential pathogens

Utilization of eight different nitrogen sources by potential pathogens of eupatorium was tested as explained in 'Material and methods' and the results are presented in the table 12.

Table 12: Dry mycelial weight of potential pathogens of *C. odorata* in different nitrogen sources

Sl.No.	Nitrogen sources	Mean dry weight (mg)		
		A	B	C
1	Ammonium nitrate	280.00	254.55	260.44
2	Asparagine	273.77	273.08	200.60
3	Methionine	210.65	106.75	158.41
4	Peptone	325.00	288.00	290.92
5	Potassium nitrate	321.67	260.00	260.61
6	Sodium nitrate	246.43	200.49	230.00
7	Tyrosine	261.00	286.93	131.92
8	Urea	258.98	166.99	113.14
	S.Em±	0.92	0.67	0.90
	CD at 1%	3.88	2.82	3.79

A = *Alternaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*

Maximum growth of *A. alternata* was recorded in peptone (325.00 mg) followed by potassium nitrate (321.67 mg) which were significantly superior over other nitrogen sources tested. Ammonium nitrate (280.00 mg) and Asparagine (273.77 mg) supported moderate growth. Urea (258.98 mg) and tyrosine (261.00 m) were on par with each other. Least growth was recorded in Methionine (210.65 mg) followed by sodium nitrate (246.43 mg).

Peptone supported maximum growth (288.00 mg) of *C. gloeosporioides,* significantly on par to tyrosine (286.93 mg). Methionine supported least growth (106.75 mg) of the pathogen. Sodium nitrate (200.49 mg) and urea supported poor mycelial growth (166.99 mg) whereas, potassium nitrate (260.00 mg), Aspargine (273.08 mg) and ammonium nitrate (254.55 mg) supported moderate growth of mycelium.

Maximum growth of *A. pullulans* was recorded in peptone (290.92 mg) which was significantly superior over other treatments. Growth was on par in potassium nitrate (260.61 mg) and ammonium nitrate (260.44 mg) and ranked second in supporting the mycelial growth. Significantly, poor growth was recorded in sodium nitrate (230.00 mg) followed by asparagine (200.60 mg), methionine (158.41 mg) and tyrosine (131.92 mg). Least growth was recorded in urea (113.14 mg).

From the results, it is clear that, peptone as nitrogen source supported maximum growth of all the three potential pathogens of eupatorium.

4.5 Toxin studies

Effect of culture filtrate of potential pathogens was studied on cuttings of eupatorium and tomato. The results of this experiment are presented. (Table 13 and Plate 7 & 8).

Table 13: Effect of culture filtrate of potential pathogens of *Chromolaena odorata* on cuttings of eupatorium and tomato

	Culture filtrate of potential pathogens	Eupatorium cuttings		Tomato cuttings	
		Symptoms	Time taken	Symptoms	Time taken
1	*Altemaria alternata*				
	a) Crude	Necrosis, drooping and wilting	24 h	Necrosis, drooping and wilting	24 h
	b) 1:1 dilution	Necrosis, drooping and wilting	36 h	Necrosis, drooping and wilting	24 h
2	*Aureobasidium pullulans*				
	a) Crude	Curling, britting complete drooping and wilting	24 h	Drooping and complete wilting	24 h
	b) 1:1 dilution	Drying of leaves and wilting	32 h	Drooping and complete wilting	32 h
3	*Colletotrichum gloeosporioides*				
	a) Crude	No symptoms	-	No symptoms	-
	b) 1:1 dilution	No symptoms	-	No symptoms	-
4	Control	No symptoms	-	No symptoms	-

Plate 7. Effect of culture filtrate of *Alternaria alternata* on eupatorium

Plate 8. Effect of culture filtrate of *Aureobasidium pullulans* on eupatorium

The results revealed that, *A. alternata* and *A. pullulans* could produce toxins and it was visualized by different symptoms produced both on tomato and eupatorium cuttings. Cuttings of tomato dipped in crude as well as in 1:1 dilution culture filtrate of *A. alternata* showed necrosis, drooping and wilting symptoms after 24 h of incubation. Similar symptoms were observed on cuttings of eupatorium dipped in crude filtrate after 24 h of incubation.

Crude and 1:1 diluted culture filtrate of *Aureobasidium pullulans* induced symptoms like drooping and complete wilting of tomato cuttings after 24 h and 32 h of incubation, respectively. Eupatorium cuttings dipped in crude extract of *A. pullulans* induced drooping, curling, brittling and wilting symptoms within 24 h of incubation. Drying of leaves, brittling and complete wilting was observed in 1:1 diluted culture filtrate after 32 h of incubation.

However, *C. gloeosporioides* could not produce any toxin and the cuttings were healthy just as in control. From this experiment, it can be concluded that crude filtrate of *A. alternata* and *A. pullulans* induced symptoms within 24 h of incubation; whereas diluted filtrate took more incubation period to produce symptoms.

4.6 Host range of potential pathogens of *Chromolaena odorata*

This experiment was carriedout to study the effect of potential pathogens of eupatorium on different economic and weed plants by following 'cut leaf assay method' as explained in 'Material and Methods'.

The plant species tested for their safety against potential pathogens of eupatorium was grouped in two categories, category A included weed plants and category B, economic plants. The results are presented in the Table 14.

Table 14: Host range of potential pathogens of *Chromolaena odorata*

Plant species	Symptoms		
	Alternaria alternata	*Colletotrichum gloeosporioides*	*Aureobasidium pullulans*
Category A			
Lantana camera	*Mild 2-3 lesions	No symptoms	No symptoms
Parthenium	No symptoms	No symptoms	No symptoms
Weed plants	No symptoms	No symptoms	No symptoms
Category B			
Economic plants			
Plantation crops			
Arecanut	No symptoms	No symptoms	No symptoms
Banana	No symptoms	No symptoms	No symptoms
Beetlevine	No symptoms	No symptoms	No symptoms
Cardamom	No symptoms	No symptoms	No symptoms
Cashew	No symptoms	No symptoms	No symptoms
Cocoa	No symptoms	No symptoms	No symptoms
Coconut	No symptoms	No symptoms	No symptoms
Coffee	No symptoms	No symptoms	No symptoms
Pepper	No symptoms	No symptoms	No symptoms
Field crops			
Cotton	No symptoms	No symptoms	No symptoms
Cowpea	No symptoms	No symptoms	No symptoms
Paddy	No symptoms	No symptoms	No symptoms
Sunflower	No symptoms	No symptoms	No symptoms
Forest trees			
Bamboo	No symptoms	No symptoms	No symptoms
Eucalyptus	No symptoms	No symptoms	No symptoms
Teak	No symptoms	No symptoms	No symptoms

* : Microscopic observation revealed the inability of pathogen to enter inside the epidermis

Category A:

***Parthenium hysterophorus* L.** : None of the potential pathogens could able to induce symptoms on this noxious weed.

***Lantana camera* L.** : On this weed only *A. alternata* was able to cause mild lesions. However, microscopic observations revealed that, pathogen failed to ramify inside the cells.

Category B:

Plantation crops : All the plantation crops tested viz., arecanut, coconut, pepper, betelvine, cardamom, cocoa, coffee, banana and cashew were very safe from the potential pathogens of eupatorium and the crops showed no symptoms.

Field crops : None of the pathogen could cause any symptoms on paddy, cotton, sunflower and cowpea, where eupatorium is an endemic weed.

Forest trees : Timber valued forest trees like teak, eucalyptus and bamboo were free from infection and hence no symptoms were noticed on them.

The results revealed that, the potential pathogens of eupatorium were very safe to use under field conditions without any adverse effect on any of the economic plants tested and hence satisfy the criteria required for a mycoherbicide.

4.7 Survey and Surveillance for endemic pathogens of eupatorium

A second year survey and surveillance (1996-97) was conducted to collect the pathogens associated with the weed and also to confirm the

endemic nature of potential pathogens selected after *in vitro* and *in vivo* screening. The pathogens collected during second year survey are presented in the table 15.

The results confirmed that, the potential pathogens of eupatorium were endemic in the areas surveyed. All the diseased samples collected from Yellapura taluk were infected by *A. alternata* (Cent per cent recovery) with typical leaf spots and veinal necrosis symptoms.

Colletotrichum gloeosporioides was proved to be endemic in Prabhunagar area with maximum recovery (90.00%). However, it was also recovered from Mundagod samples but with low recovery (10.00%). It was noticed that, some samples collected from Dharwad local were also infected with *A. alternata. Aureobasidium pullulans* was found to be confined in its distribution to Mundagod taluk with its maximum recovery (90.00%) and caused severe infection of black mould on flowers of eupatorium

From the present experiment, it can be concluded that, *A. alternata, Aureobasidium pullulans* and *C. gloeosporioides* were endemic pathogens of eupatorium and hence they appear to be promising candidates for mycoherbicidal development.

4.8 Standardization of inoculation techniques

To know the best method of inoculation for maximum disease incidence on eupatorium, different inoculation methods were tried as explained in 'Material and Methods' and the results are presented in the Table 16.

None of the pathogens were successful in initiating the disease symptoms on the weed either by seed or soil inoculation. Similarly, stem

Table 15: Survey and surveillance for diseases of *Chromolaena odorata* (1996-97)

Sl. No.	Location	Pathogens isolated	No. of diseased leaves / flowers	Per cent recovery of pathogens
1.	Dharwad			
	a. Dharwad	*Colletotrichum gloeosporioides*	50	80
		Alternaria alternata	50	20
	b. Prabhunagar	*Colletotrichum gloeosporioides*	50	90
2.	Mundagod	*Colletotrichum gloeosporioides*	50	10
		Aureobasidium pullulans	50	90
3.	Yellapur	*Alternaria alternata*	50	100

Table 16: Effect of different inoculation techniques on the development of disease in *Chromolaena odorata*

Sl. No.	Method / Technique	Pathogen		
		A	B	C
1	Seed inoculation	-	-	-
2	Soil inoculation	-	-	-
3	Stem inoculation	-	-	-
4	Foliage inoculation	+	+	Poor infection*
5	Blossom inoculation	-	-	+

+ : Symptom expression
- : No symptoms
* : The pathogen caused only few mild lesions
A : *Alternaria alternata*
B : *Colletotrichum gloeosporioides*
C : *Aureobasidium pullulans*

inoculation technique was not effective and the plants inoculated with pathogens showed no symptoms.

The results were positive when the pathogens were inoculated to foliage / flowers by spray inoculation technique. Leaf spot symptoms produced by foliar pathogens viz., *A. alternata* and *C. gloeosporioides* were prominent. However, *A. pullulans* induced very minute lesions on the foliage.

Blossom infection was noticed only in inoculation of *A. pullulans.* Two foliar pathogens failed to induce any symptoms on flowers. *A. pullulans* produced black mould of inflorescence. The results indicated that, spraying was the only technique to cause maximum disease incidence on the weed plants.

4.8.1 Effect of inoculum density of pathogens on eupatorium

The experiment was conducted to standardize the optimum inoculum threshold of potential pathogens to induce maximum disease on eupatorium. A wide range of spore concentration (10^2 to 10^{20} spores/ml) was tested on eupatorium as explained in 'Material and Methods' and the results are presented (Table 17 and Fig. 4).

Alternaria alternata caused maximum leaf spot disease (72.02 per cent disease index) at a spore concentration of 10^{10} spores per ml followed by 10^{12} spores/ml with 70.13 per cent disease index. Infection level was decreased drastically after 10^{12} spores/ml spore concentration. Per cent disease index was 20.00 and 25.00 at 10^{18}, 10^{16} and 10^{14}, respectively. Infection was completely ceased above 10^{18} spores per ml.

Maximum leaf spot (82.12 per cent disease index) due to *C. gloeosporioides* was recorded at 10^{14} spores per ml spore concentration.

Table 17: Effect of inoculum density of potential pathogens on disease severity of *Chromolaena odorata*

Sl. No.	Spore concentration (No/ml)	Disease severity				
		A		B		C
		Disease grade	Per cent disease index	Disease grade	Per cent disease index	Grade
1	0	0	0	0	0	0
2	10^{2}	0	0	0	0	0
3	10^{4}	1	1.00	0	0	0
4	10^{6}	2	14.21	3.00	0	0
5	10^{8}	4	38.92	3	24.19	0
6	10^{10}	5	72.02	4	43.00	Low
7	10^{12}	5	70.13	4	55.04	Low
8	10^{14}	3	25.00	5	82.12	Moderate
9	10^{16}	3	20.00	5	75.63	Maximum
10	10^{18}	3	20.00	5	75.00	Maximum
11	10^{20}	0	0	3	18.18	Maximum

A = *Altemaria alternata*
B = *Colletotrichum gloeosporioides*
C = *Aureobasidium pullulans*
Low = ¼ of the inflorescence infected
Moderate = ½ of the inflorescence infected
Maximum = whole inflorescence infected

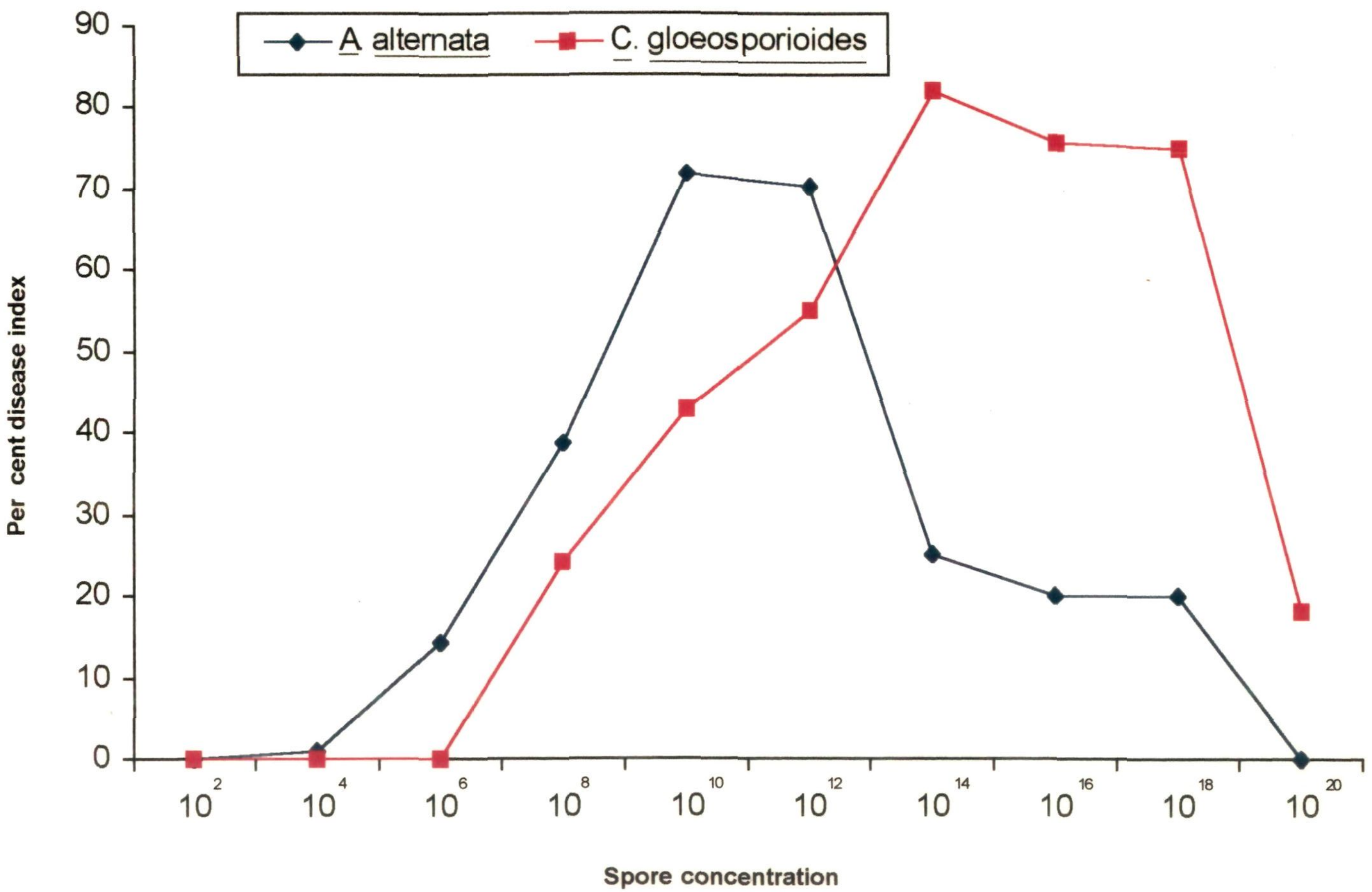


Fig. 4: Effect of inoculum density on disease severity of *Chromolaena odorata*

Infection level did not increase even with the increase in spore concentration. However, infection was gradually increased as the spore concentration increased from 10^6 spores per ml to 10^{14} spores per ml. Infection was least at 10^{20} spores/ml with per cent disease index of 18.18.

Lower inoculum densities of *Aureobasidium pullulans* failed to cause flower black mould. At spore concentration of 10^2 to 10^8 spores per ml, black mould development was nil. Infection level was low to moderate at 10^{10} to 10^{14} spores per ml and it increased gradually with increase in inoculum concentration. Disease was maximum at a spore concentration of 10^{16} to 10^{20} spores per ml. Therefore, 10^{16} spores per ml was standardized as optimum spore dose of *A. pullulans* for maximum disease development.

From the results it is clear that, standardized spores load for *A. alternata, C. gloeosporioides* and *A. pullulans* was 10^{10} spores per ml, 10^{14} spores per ml and 10^{16} spores per ml, respectively. In further experiments standardized spore load of each pathogen was maintained.

4.8.2 Identification of vulnerable stage of the weed for maximum infection

To induce maximum infection on the weed, the pathogen should be inoculated at their optimum spore concentration at crucial stage of the weed. The pathogens were sprayed on weed plants at different growth stages as explained in 'Material and Methods' and susceptible stage was identified based on disease intensity (Table 18a & 18b and Fig. 5).

The data revealed that, young weed plants at two months age were more susceptible to *C. gloeosporioides,* with maximum per cent disease index of 83.46. As the plants aged, their susceptibility towards *C. gloeosporioides* was

Table 18a: Effect of age of *Chromolaena odorata* on severity of the disease

Sl. No.	Plant age	Disease severity				
		A		B		C
		Disease grade	Per cent disease index	Disease grade	Per cent disease index	Grade
1	One month old (June-July)	-	-	-	-	-
2	Two months old (July-August)	1	3.00	5	83.46	-
3	Three months old (August-September)	5	70.51	4	55.11	-
4	Four months old (September-October)	5	65.65	2	15.00	-
5	Five months old (October-November)					
	a) Foliage	2	10.15	1	2.00	+ mild lesions
	b) Flower	-	-	-	-	+
6	Flowering plants (Flowers)	-	-	-	-	+

+ : Infection due to *A. pullulans*
- : No symptoms
A : *Alternaria alternata*
B : *Colletotrichum gloeosporioides*
C : *Aureobasidium pullulans*

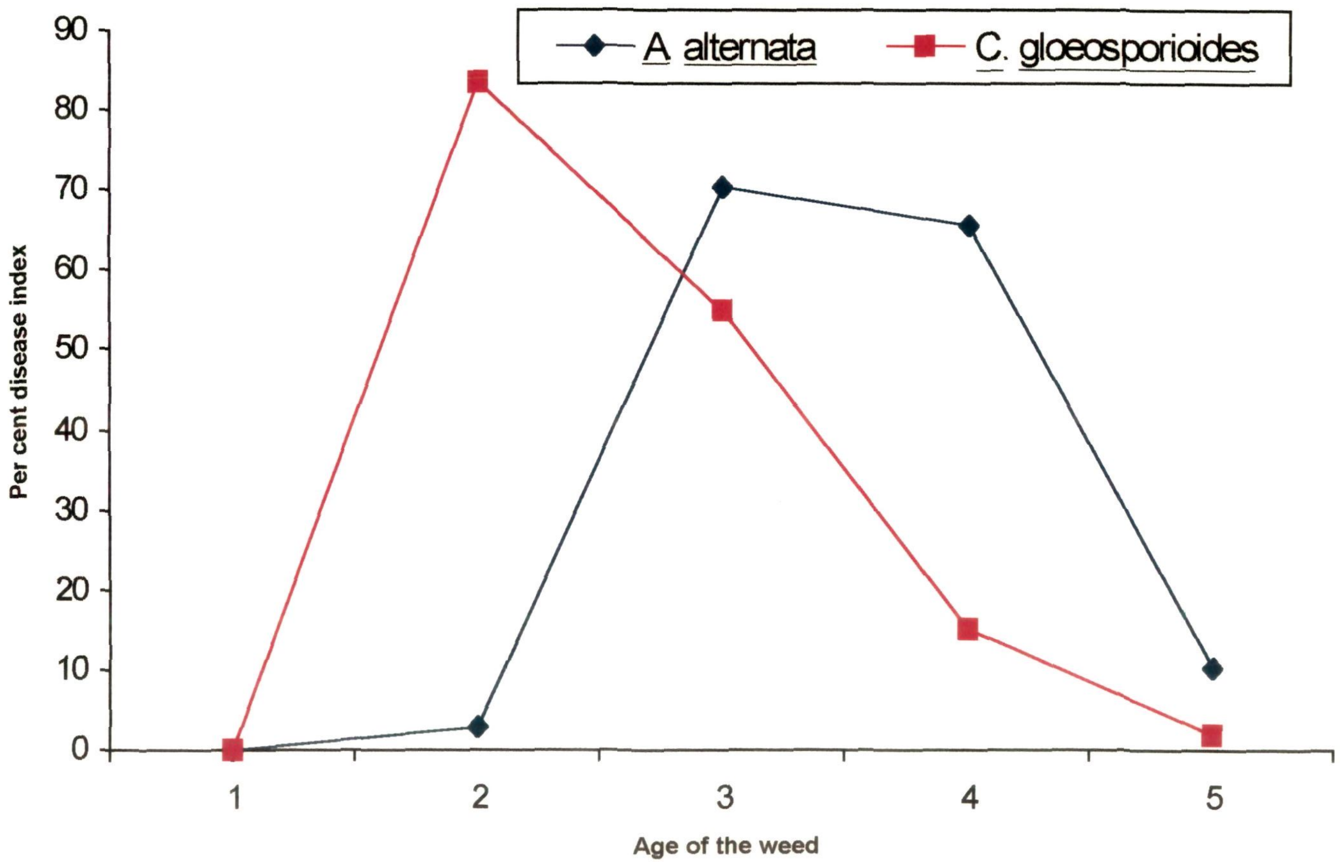


Fig. 5: Effect of age of *Chromolaena odorata* on disease severity

decreased and the infection was minimum on foliage of five months old plants.

Alternaria alternata was able to infect three months old weed plants, which caused 70.51 per cent disease index. On aged plants, leaf spots were noticed mainly on older leaves and plants at 4-5 months age were less susceptible to infection. Per cent disease index was least (10.15) on five months old plants.

Plants at flowering stage were infected with *Aureobasidium pullulans*. It produced few minute lesions on leaves and black mould like growth on flowers.

4.8.3 Effect of *Aureobasidium pullulans* on flowers of eupatorium

Aureobasidium pullulans was inoculated on flowers at different stages of development as explained in 'Material and Methods'. The results revealed that, bud and flower initiation stages were immune to infection. Infection was noticed only at flower opening stage onwards with varying degrees.

Aureobasidium pullulans inoculated at flower opening stage resulted in black mould like growth of entire flower and dropping of immature buds was noticed (Plate 9). Maximum infection was noticed even at full bloom stage but the degree of infection varied. It resulted in black mould like growth on flowers at full bloom stage and seeds were sterile and abnormal. Infection after fertilization and at seed maturity stage was superficial and minimum. Matured seeds were resistant to infection.

From the results, it was evident that, spraying of *C. gloeosporioides* on two months old weed plants and subsequent spray inoculation with *A. alternata* at three months stage resulted in the maximum disease incidence on foliage.

Table 18b: **Effect of *Aureobasidium pullulans* on different reproductive stages of *Chromolaena odorata***

Sl. No.	Reproductive stages of the weed	Infection	Parts affected
1	Bud initiation	-	-
2	Flower initiation	-	-
3	Flower opening	++	Entire flower bud
4	Full bloom	++	Flower and developing seed
5	After fertilization and at seed maturity	+	Infection superficial and matured seeds resistant

++ : Maximum infection
+ : Lower infection
- : No infection

Plate 9. Infection of *Aureobasidium pullulans* on blossom (*In vitro*)

Spraying of *A. pullulans* on just opening flowers and at full bloom resulted in maximum black mould of flowers and sterile, abnormal seeds.

4.8.4 Time requirement for symptom expression and pathogenesis of potential pathogens on eupatorium

Pathogenesis and time requirements for symptom expression are presented in the table 19a & 19b.

Alternaria alternata caused leaf spots characterised by target board effect. Spots measured upto 1-2 cm and surrounded by yellow halo. Later, spots coalesced resulting in drying of leaves and defoliation. However, symptoms were more pronounced on older leaves. On some young branches veinal necrosis was also observed. Some of the plants sprayed with *A. alternata* exhibited drooping and wilting symptoms. Symptoms due to *A. alternata* was noticed on twelfth day after spraying (Plate 10, 11 & 12).

Colletotrichum gloeosporioides caused leaf spots which were black, irregular and minute to large, distributed in the centre of leaf lamina or on the margin. Under humid conditions, the spots enlarged and coalesced to farm large patches. The affected portion turned brittle and resulted in amputation of the same. Thus, several shot holes were observed on affected leaves. The symptoms were noticed more on young leaves than older ones. The pathogen required ten days of incubation period for symptom expression (Plate 13 & 14).

Aureobasidium pullulans infected flowers and caused black mould like growth covering the entire inflorescence. It invaded the developing flowers and immature seeds and resulted in sterile seed production. Pathogenesis of *A. pullulans* on flower buds resulted in premature dropping of buds and some times resulted in malformed seeds. The symptoms were noticed eight days after

Plate 10. Infection of *Alternaria alternata* on older leaves

Plate 11. Leaves infected with *Alternaria alternata*

Plate 12. Drooping and wilting of eupatorium seedlings due to *Alternaria alternata* spraying

Table 19a: Pathogenesis caused by potential pathogens of *Chromolaena odorata*

	Pathogens	Pathogenesis
1.	*Alternaria alternata*	a) Leaf spots with target board symptoms b) Later coalesced, drying and defoliation. More severe on lower leaves (older) c) Veinal necrosis, drooping and wilting of plants
2.	*Aureobasidium pullulans*	a) Black moulds on flowers b) Premature drying of flowers c) Malformed seeds, reduced seed germination and sterile seeds
3.	*Colletotrichum gloeosporioides*	a) Leaf spots b) Leaf drying and defoliation c) Shot hole symptoms

Table 19b: Number of days for symptom expression on *Chromolaena odorata*

	Pathogens	No. of days for symptoms expression
1	*Alternaria alternata*	12
2	*Aureobasidium pullulans*	8
3	*Colletotrichum gloeosporioides*	10

Plate 13. Eupatorium leaves showing infection of ***Colletotrichum gloeosporioides***

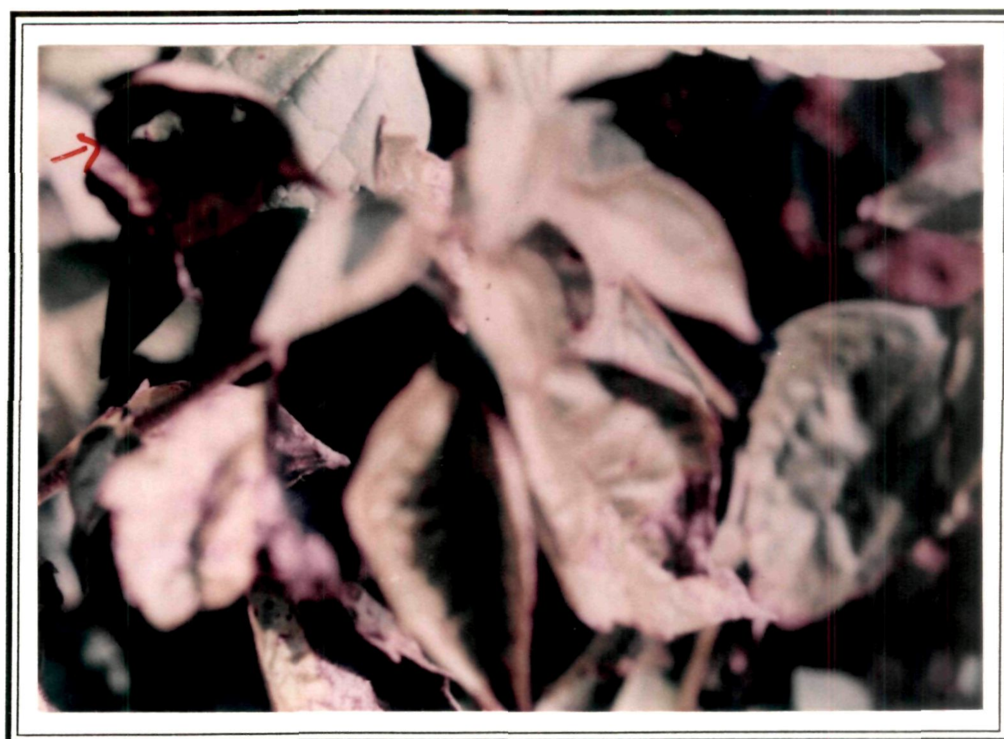


Plate 14. Shot hole symptoms on eupatorium leaves due to ***Colletotrichum gloeosporioides*** **infection**

spraying. However, mild lesions on leaves were noticed fifteen days after spraying.

4.8.5 Histopathological studies:

The histopathological study was carried out to know the changes that occurred at cellular level due to penetration of the pathogens.

The observation revealed that the epidermal cells and mesophyll cells of healthy leaves were compact and the vascular bundles were normal (Plate 15).

In Infected leaf, the penetration of the pathogens took through the stomatal opening and directly. Infection resulted in destruction of lower and upper epidermal cells. Cell walls of mesophyll cells were damaged. Infection resulted in disruption of spongy and palisades layer and thickness of leaves was reduced. Infected vascular bundles were necrotic (Plate 16, 17 & 18).

Infection of *A. pullulans* on flowers resulted in direct colonization and destruction of reproductive parts (Plate 19).

4.9 Combined effect of potential pathogens on eupatorium

All the three potential pathogens were inoculated individually and in all possible combinations on eupatorium as explained in 'Material and Methods' to know whether the interaction between the pathogens was synergistic or antagonistic. The results are presented in the table 20.

From the results, it is clear that, the interaction among the potential pathogens was synergistic. Disease was maximum in combination treatments than in individual treatments. Disease was significant in T_7 i.e., combination of all pathogens *viz., A. alternata + C. gloeosporioides + A. pullulans* (94.00 per

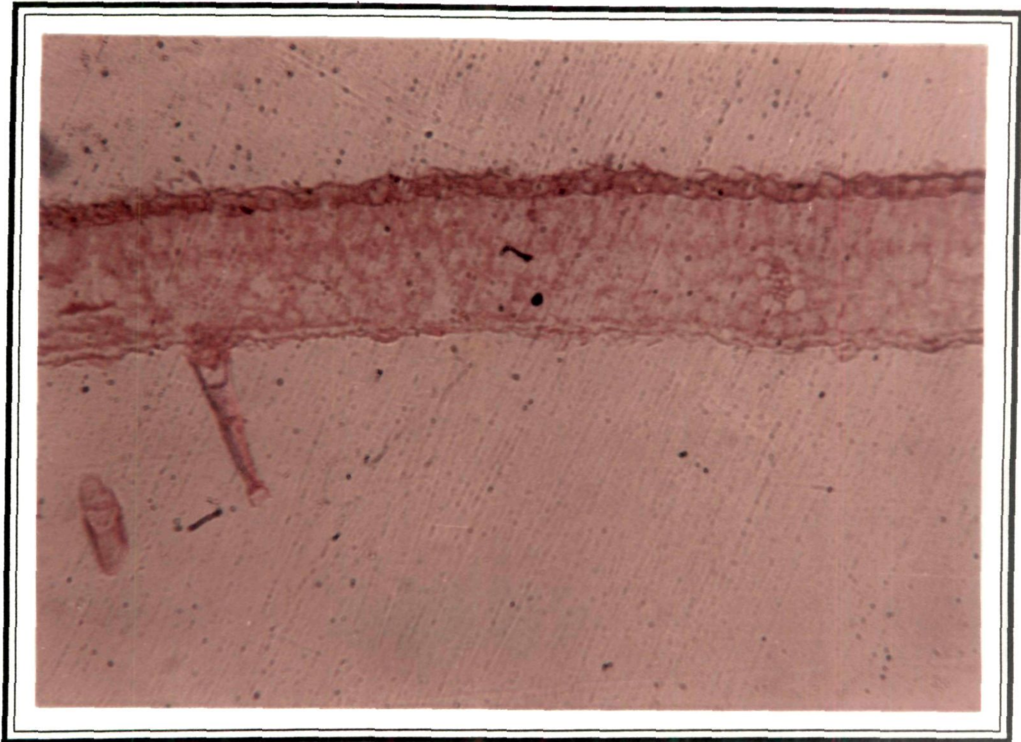
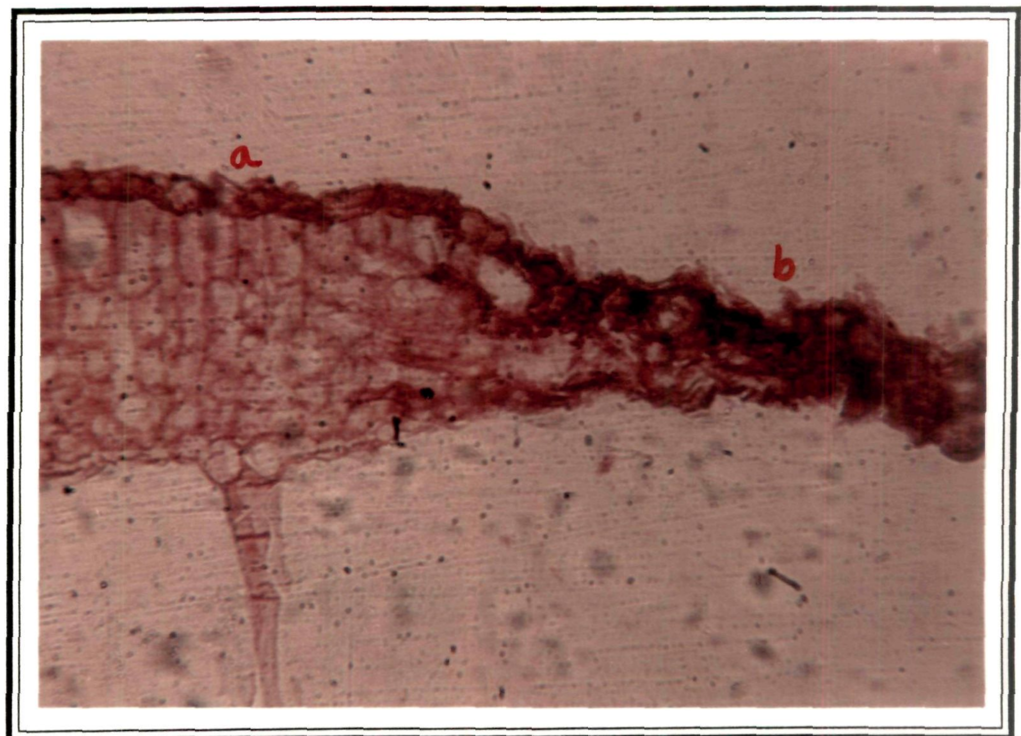


Plate 15. Healthy eupatorium leaf with intact epidermis mesophyll and palisade layers



a. Healthy part, b. Diseased part

Plate 16. Histopathology of eupatorium leaf

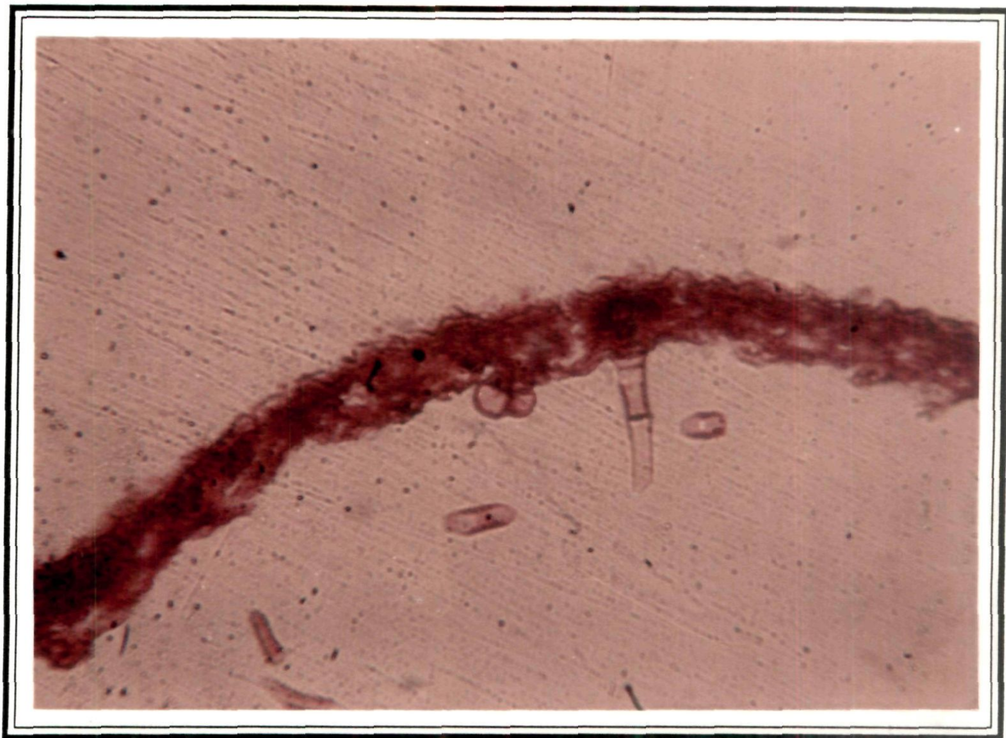


Plate 17. Infected leaf showing reduced leaf thickness

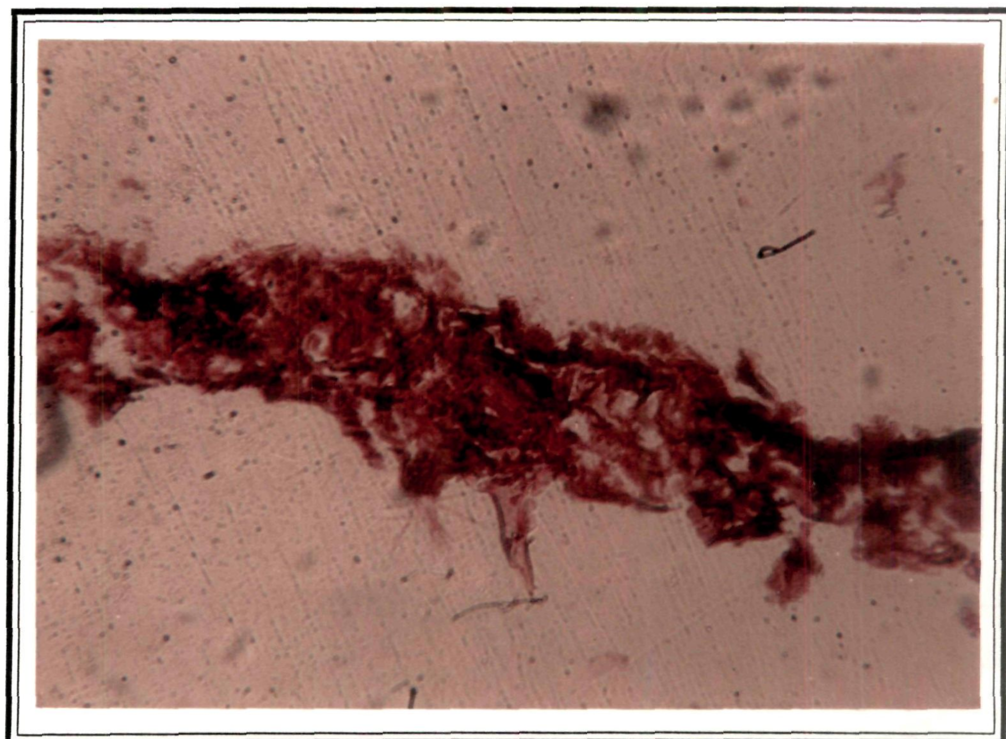
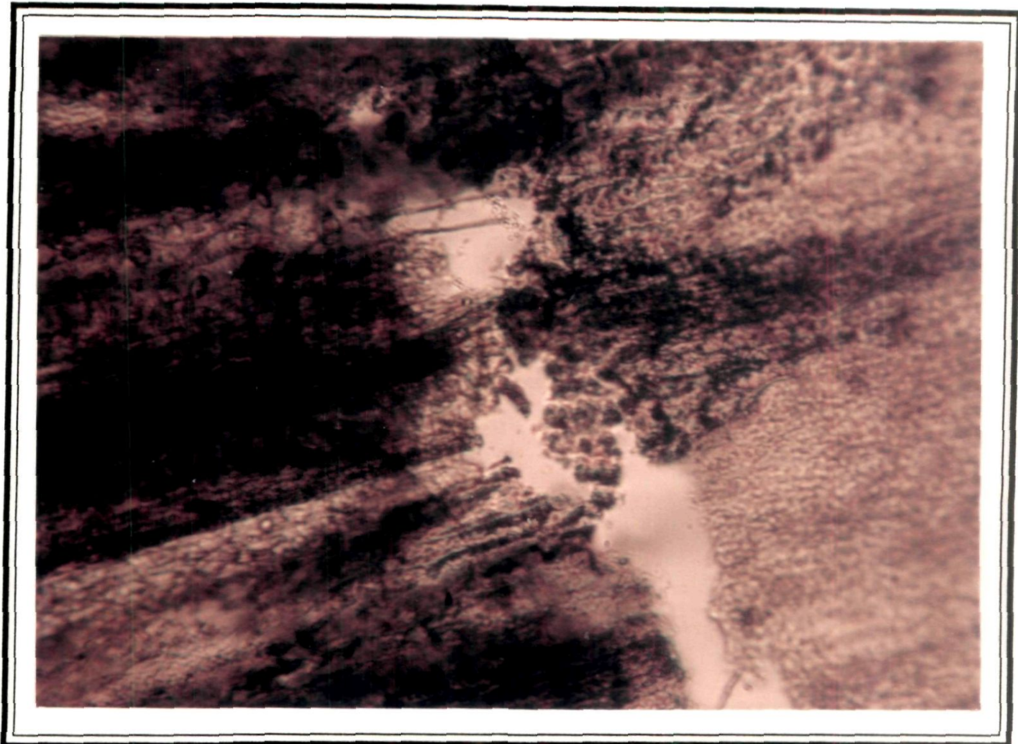
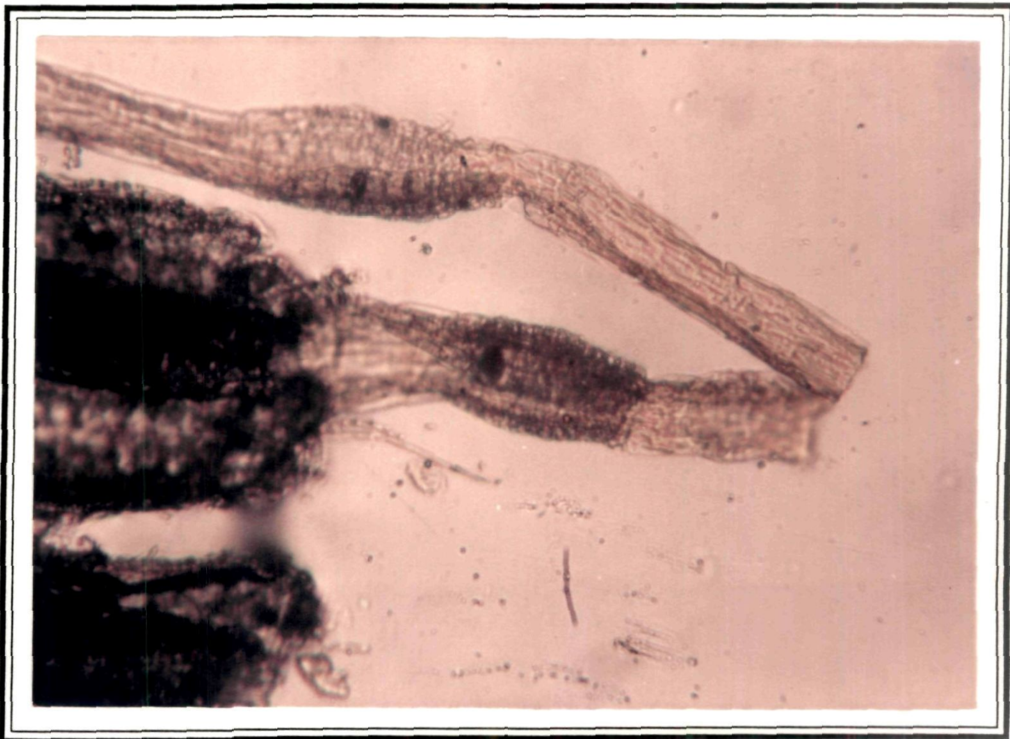


Plate 18. Diseased leaf with damaged mesophyll, epidermis and palisade layers



a. Colonization and spread of mycelium



b. Infected anther lobe

Plate 19. Cross section of diseased single flower infected with *Aureobasidium pullulans*

Table 20: Synergistic effect of potential pathogens on *Chromolaena odorata*

	Pathogens	Per cent disease index	No. of days for symptom expression
1	*Alternaria alternata* (Foliage)	60.00 (50.77)*	12
2	*Colletotrichum gloeosporioides* (Foliage)	80.00 (63.44)	10
3	*Aureobasidium pullulans* (Flowers)	100.00 (90.00)	8
4	*A. alternata* + *C. gloeosporioides* (Foliage)	92.00 (73.57)	8
5	*A. alternata* + *A. pullulans* (Foliage)	65.00 (53.73)	12
6	*A. pullulans* + *C. gloeosporioides* (Foliage)	82.00 (65.12)	10
7	*A. alternata* + *C. gloeosporioides* + *A. pullulans* (Foliage)	94.00 (75.82)	8
8	Control (Water spray)	-	-
	S.Em±	6.58	-
	CD at 1%	20.25	

*Figures in parenthesis indicate arc sine transformed value

cent disease index) followed by combination of two foliar pathogens i.e., *A. alternata* + *C. gloeosporioides* (92.00 per cent disease index). In combination treatments, incubation period for symptom expression was also shortened (eight days) compared to their individual performance (ten or twelve days). Combination treatments were significantly superior over individual spray inoculation of *A. alternata* or *C. gloeosporioides* (60.00 and 80.00 per cent disease index respectively).

Aureobasidium pullulans alone caused cent per cent disease on flowers after eight days of inoculation. However, combination of *A. pullulans* either with *A. alternata* or *C. gloeosporioides*, showed poor synergism, with slight increase in PDI (65.00 and 82.00) compared to their individual performance (60.00 and 80.00 per cent disease index, respectively).

From this experiment, it is clear that spray inoculation of two foliar pathogens in combination, *A. alternata* @ 10^{10} spores per ml + *C. gloeosporioides* @ 10^{14} spores/ml was best to enhance the foliar disease pressure and to advance the disease initiation under glasshouse conditions.

4.10 Mass production of potential pathogens

Stock culture

Agar slants were used as stock cultures for all the potential pathogens and they were stored at -4°C.

Selection of culture media

Several agar media were tested to findout the best culture medium as explained in 'Material and Methods'. Since all the pathogens grew and sporulated abundantly on PDA, it was selected as best culture medium.

Mass production of potential pathogens

Methodology was developed to produce mycoherbicides using submerged and solid substrate fermentation techniques. The results of the experiment are presented in the form of flow charts for each pathogen (Flow chart 1, 2 & 3).

For mass production of potential pathogens different substrates viz., eupatorium leaf extract + 1 per cent sucrose, eupatorium leaf bits and potato dextrose broth were tested for their ability to induce sporulation and growth of the pathogens. The results are tabulated in the Table 21. The results revealed that, *A. alternata* sporulated fairly on both eupatorium leaf extract agar and eupatorium leaf bits. However, maximum sporulation was observed in potato dextrose broth. Hence, PDB was selected for mass production of *A. alternata* and the technique employed was submerged fermentation technique.

The *C. gloeosporioides* sporulated heavily on all the substrates tested and hence, cheaply available eupatorium leaf bits were used in mass production. The technique employed was solid substrate fermentation.

Aureobasidium pullulans showed excellent sporulation and growth on all the three substrates tested. But, it was noticed that, colonization and sporulation was very fast on eupatorium leaf bits for maximum sporulation when compared to other substrates. Hence, *A. pullulans* was mass produced on sterilised leaf bits and the methodology employed was solid substrate fermentation technique.

4.10.1 Mass production of *Alternaria alternata* (Submerged fermentation)

The *A. alternata* was mass produced in potato dextrose broth and production fermentation was extended upto fifteen days at room temperature. The growth was collected and whole broth was homogenised. After harvest,

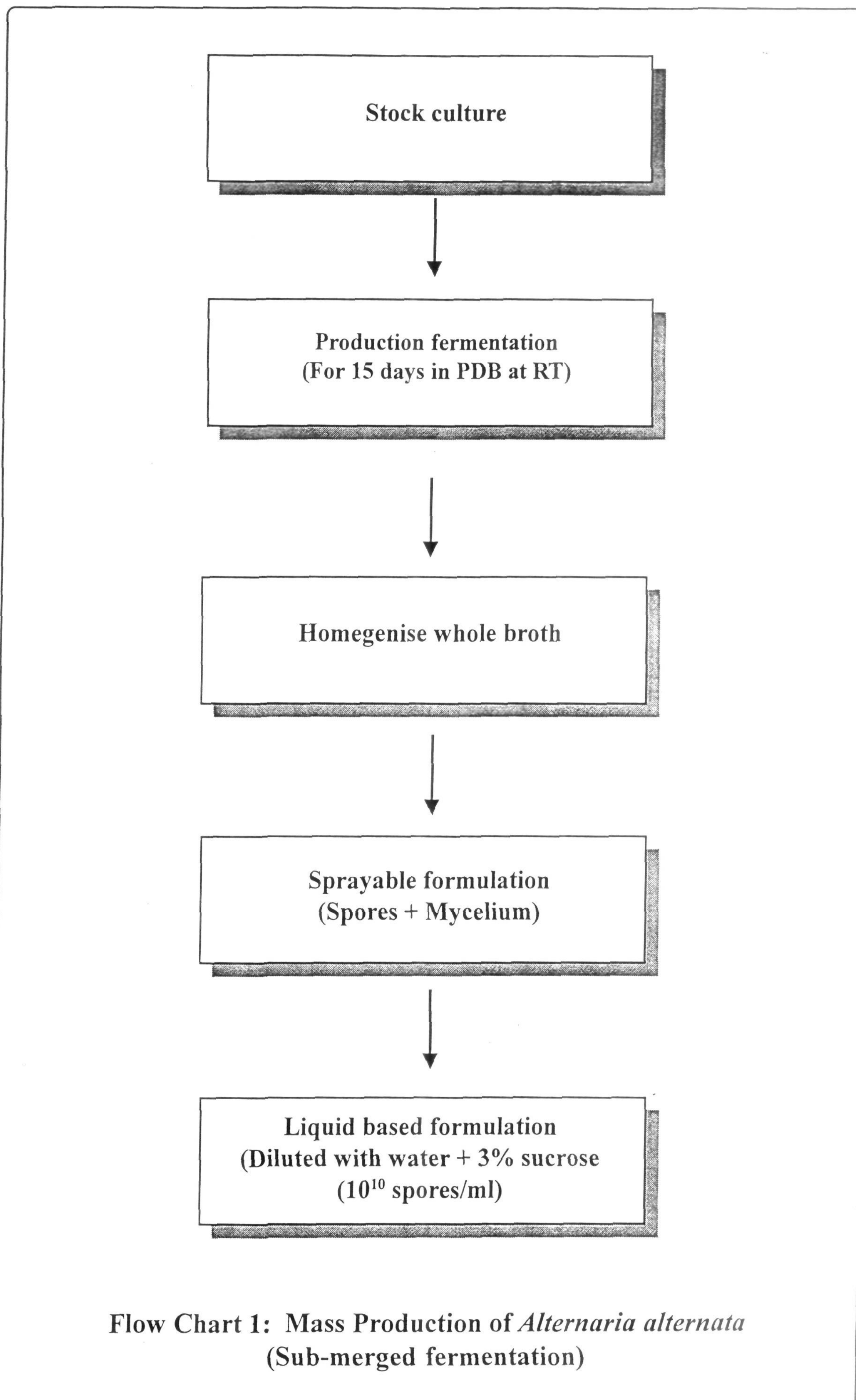
Table 21: Sporulation capacity of potential pathogens on different substrates used in mass production

Substrates	Sporulation capacity		
	Pathogens		
	A	B	C
Eupatorium leaf extract + 1% sucrose	++	++	++
Sterilized Eupatorium leaf bits	++	+++	+++
Potato dextrose broth	+++	+++	+++

A : *Alternaria alternata*
B : *Aureobasidium pullulans*
C : *Colletotrichum gloeosporioides*

Rate of sporulation

++ : Good (50-70 spores/ microscopic field
+++ : Excellent (>70 spores/microscopic field)



Flow Chart 1: Mass Production of *Alternaria alternata*
(Sub-merged fermentation)

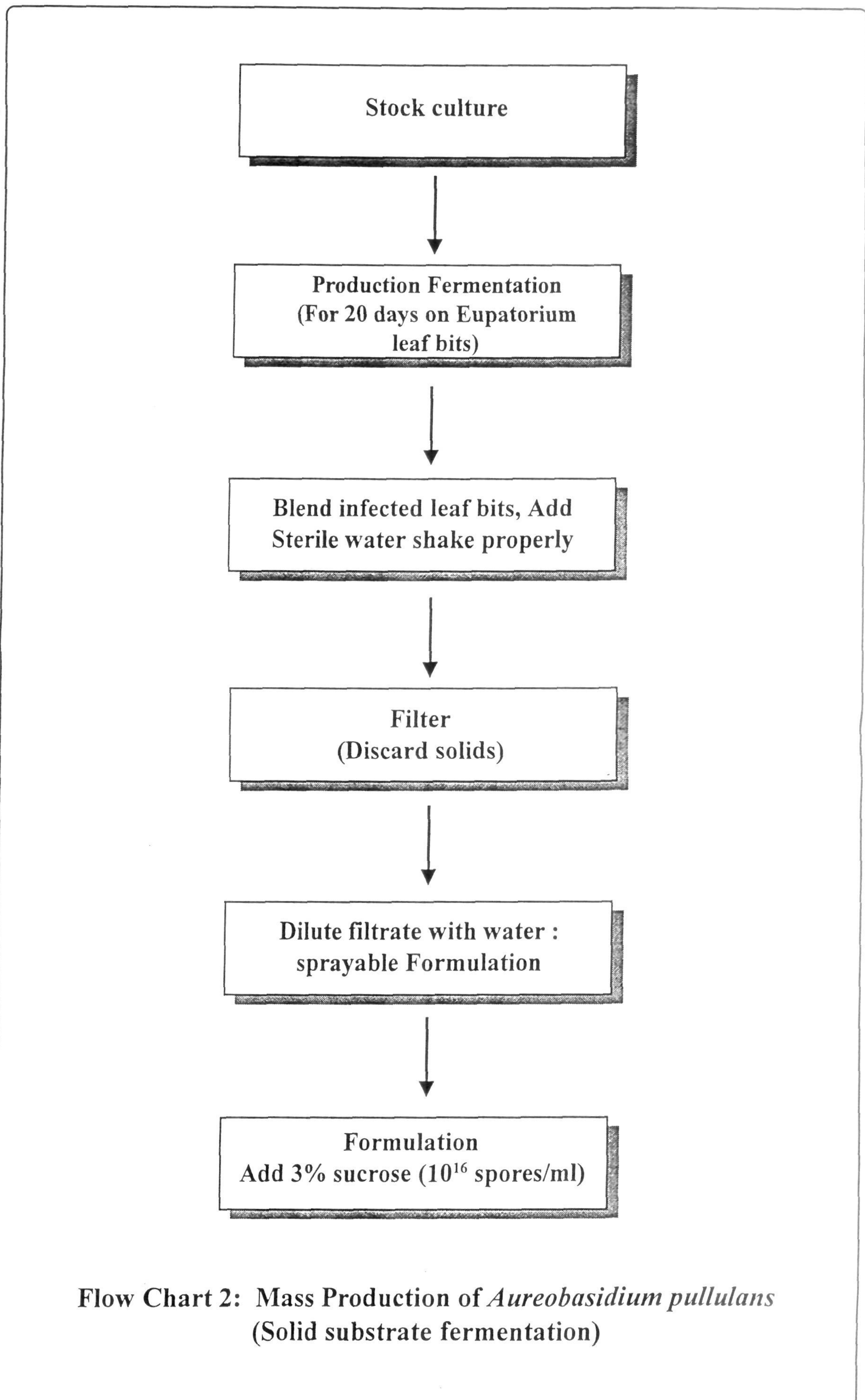
the growth (spores + mycelium) was formulated into a sprayable material. The suspension was diluted with the universal carrier i.e., water and spore dose (10^{10} spores/ml) was adjusted before spraying. Spray additive 3 per cent sucrose (w/v) was added and sprayed on foliage of eupatorium as explained in 'Material and Methods' (Flow chart 1).

4.10.2 Mass production of *Aureobasidium pullulans* (Solid substrate fermentation)

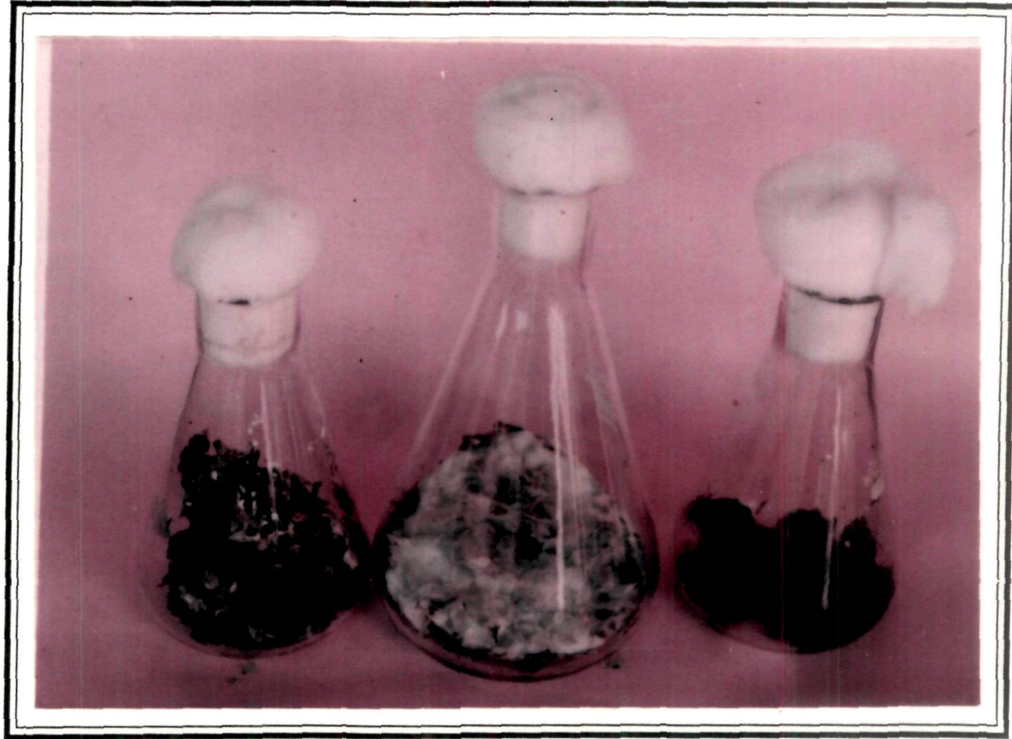
Aureobasidium pullulans was mass produced by solid substrate fermentation as explained in 'Material and Methods'. After production fermentation, the volume of leaf bits was reduced to one fourth volume due to heavy colonization and sporulation. Further, it was mixed with little quantity of water and blended in a blender. The suspension was filtered and solids were discarded. The suspension was diluted with water and mixed with 3 per cent sucrose (w/v). Before spraying, spore load (10^{16} spores/ml) was verified and sprayed on flowers of eupatorium (Flow chart 2). Spore load included all types of spores produced by *A. pullulans viz.,* blastospores, conidia and chlymadospores (Plate 20).

4.10.3 Mass production of *Colletotrichum gloeosporioides* (Solid substrate fermentation)

Colletotrichum gloeosporioides was mass multiplied by solid substrate fermentation technique for twenty days on sterilized eupatorium leaf bits. Further procedure was same as that of *A. pullulans* mass production. After twenty days of production fermentation, it was mixed with little water and blended. The suspension was filtered and solids were discarded. The filtrate contained spores and few hypal bits. The spore load was adjusted to 10^{14}

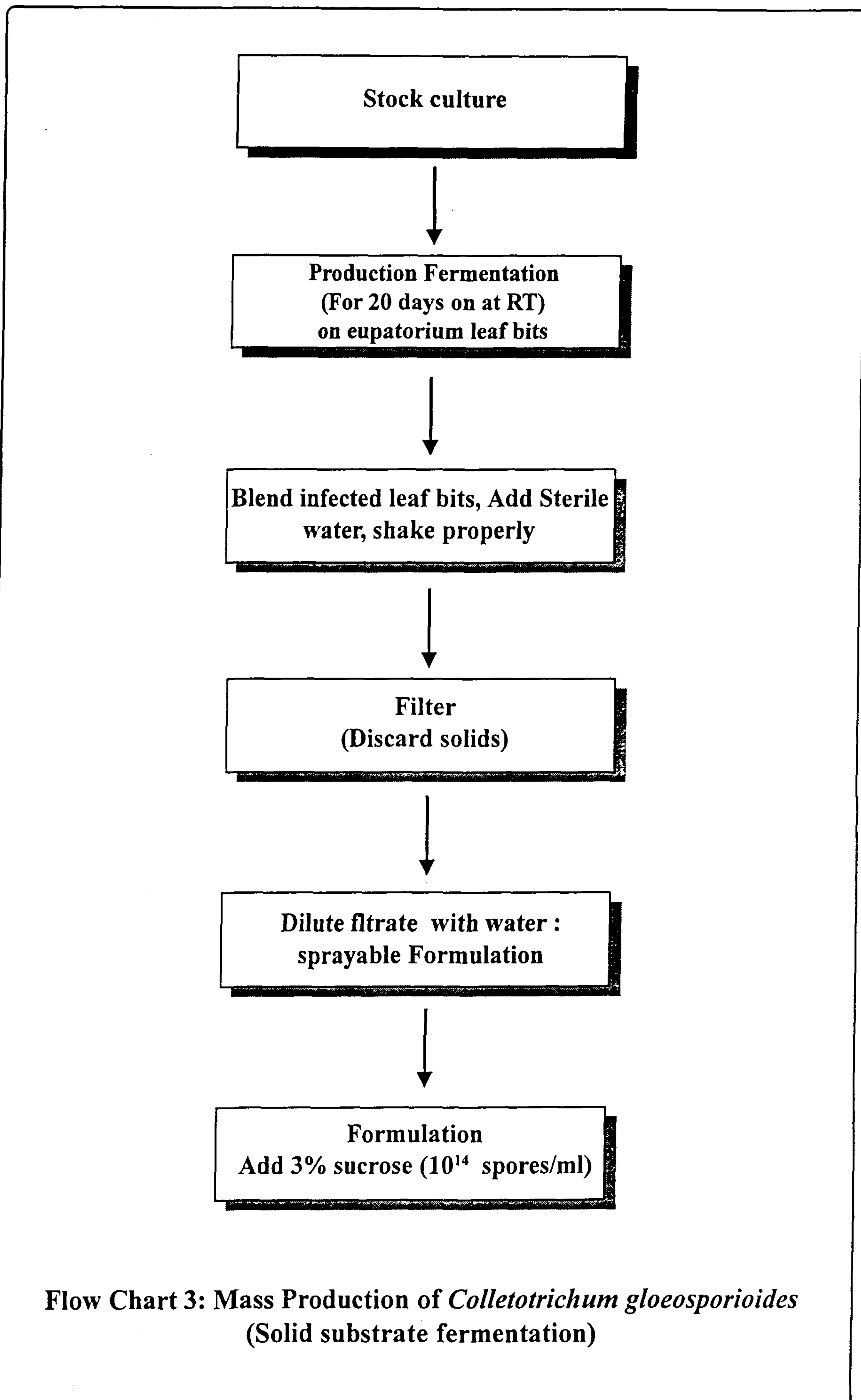


Flow Chart 2: Mass Production of *Aureobasidium pullulans*
(Solid substrate fermentation)



a. Healthy eupatorium leaves, b. Colonisation and mulitplication of the pathogen
c. Reduction in soil substrates (1/4th) quantity

Plate 20. Mass production of *Aureobasidium pullulans* on eupatorium leaf bits



Flow Chart 3: Mass Production of *Colletotrichum gloeosporioides* (Solid substrate fermentation)

spores/ml by mixing with water and 3 per cent sucrose (w/v) was added before spraying (Flow chart 3).

Such mass multiplied pathogens were sprayed on weed plants at susceptible stage to create artificial epiphytotics. As spraying of foliar pathogens was coincided with rainy season and accomplished during evening time, there was no need to maintain extra dew periods. However, 48 h dew period was maintained to get maximum infection of *A. pullulans*, as there was low rainfall coupled with low relative humidity during flowering season i.e., November and December.

4.10.4 Influence of spray additives on disease severity

The experiment was conducted to know the effect of water and 3% sucrose (w/v) additives in advancing the disease epiphytotics. The results are presented in the Table 22.

The results indicated that, number of days required for disease severity due to *A. alternata, A. pullulans, c. gloeosporioides* and *A. alternata* + *C. gloeosporioides* combination sprayed in water was 28, 25, 26 and 23 days, respectively. Addition of 3% sucrose (w/v) to spray solution always advanced the period of disease severity, which ranged from 3 to 5 days.

The results indicated that, inoculation of pathogens with 3 per cent sucrose (w/v) was better over spraying only with water.

4.11 Field efficacy of potential pathogens of eupatorium on disease severity

To test the field performance of potential pathogens they were inoculation individually and also in combinations on eupatorium weed.

Table 22: Effect of spray additives on disease development (No. of days) on *Chromolaena odorata*

Sl.No.	Pathogens	No. of days for disease development		
		Water	3% sucrose (w/v) + water	Dew period (h)
1.	*Alternaria alternata* (Foliar spray)	28	25	-
2.	*Aureobasidium pullulans* (Floral spray)	25	20	48 h
3.	*Colletotrichum gloeosporioides*	26	22	-
4.	*Alternaria alternata* + *C. gloeosporioides* (Foliar spray)	23	20	-

- = No dew period maintained

Aureobasidium pullulans was inoculated on just opening inflorescence and on full bloom during November or December. Foliar pathogens *viz., A. alternata* and *C. gloeosporioides* were sprayed on two and half months old plants, during mid August and all foliar inoculations followed this schedule. The results are presented in Table 23a.

The results revealed that, all the treatments were significantly effective in damaging eupatorium. Foliar pathogens, *Alternaria alternata* and *C. gloeosporioides* caused disease epiphytotics after 24 days and 21 days of inoculation with PDI 56.22 and 70.33 respectively.

On flowers, cent per cent infection of black mould by *Aureobasidium pullulans* was recorded and epiphytotic was created after 18 days of spraying (Plate 21).

Among the combination treatments, foliar spray inoculation of *A. alternata* + *C. gloeosporioides* + *A. pullulans* (T_7) was significantly superior over all other treatments. It resulted in maximum PDI 85.98. Treatment T_4 i.e., combination of two foliar pathogens *A. alternata* + *C. gloeosporioides* was significantly on par with T_7, which caused 83.36 per cent disease index. However, time required for artificial epiphytotics was twenty days in both the treatments.

Combination of *A. pullulans* either with *A. alternata* or *C. gloeosporioides* had very less influence on disease epiphytotics, as revealed by poor increase in PDI i.e., from 56.22 to 60.00 and 70.33 to 72.67,k respectively.

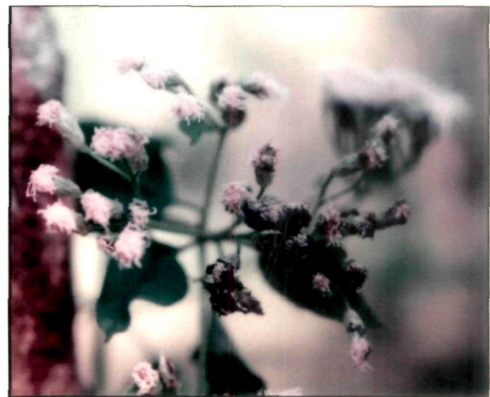
The results indicated that, foliar spray inoculation of *A. alternata* + *C. gloeosporioides* exerted maximum disease pressure on eupatorium, which resulted in epiphytotics after twenty days of inoculation. Spraying of

Table 23a: Effect of spraying of potential pathogens on per cent disease incidence and epiphytotic development on *Chromolaena odorata*

	Pathogens	PDI	Time required for epiphytotics (days)
1	*Alternaria alternata* (Foliage)	56.22 (48.56)*	24
2	*Colletotrichum gloeosporioides* (Foliage)	70.33 (56.98)	21
3	*Aureobasidium pullulans* (Flowers)	100.00 (90.00)	18
4	*A. alternata* + *C. gloeosporioides* (Foliage)	83.36 (70.00)	20
5	*A. pullulans* + *A. alternata* (Foliage)	60.00 (50.77)	24
6	*A. pullulans* + *C. gloeosporioides* (Foliage)	72.67 (58.44)	20
7	*A. pullulans* + *A. alternata* + *C. gloeosporioides* (Foliage)	85.98 (71.47)	20
8	Control (Water + 3% sucrose)	0.00 (0.00)	0
	S.Em±	20.56	-
	CD at 1%	9.56	-

*Figures in parenthesis indicate arc sine transformed value

Stage 1



Stage 2

Stage 3

Stage 4

Plate 21. Stages of infection of *Aureobasidium pullulans* on flowers

A. pullulans on flowers at opening and full bloom stage resulted cent per cent infection of inflorescence. Epiphytotics were created after 20 days of spraying.

4.11.1 Impact of *Aureobasidium pullulans* on seed development and seed viability

From the diseased flowers, cypsella were collected and further tested for their viability as explained in 'Material and Methods'. The results are presented in the Table 23b and Plate 22. Infection of *A. pullulans* on just opening flowers resulted in either premature drying of flowers or complete destruction of flower parts. Infection at full bloom stage resulted in sterile and malformed seeds. Healthy seeds recorded from infected flowers were negligible. In general, *A. pullulans* was potential to destruct seed development and produce sterile and malformed seeds.

4.12 Physiological, biochemical and biophysical changes in eupatorium due to infection under field conditions

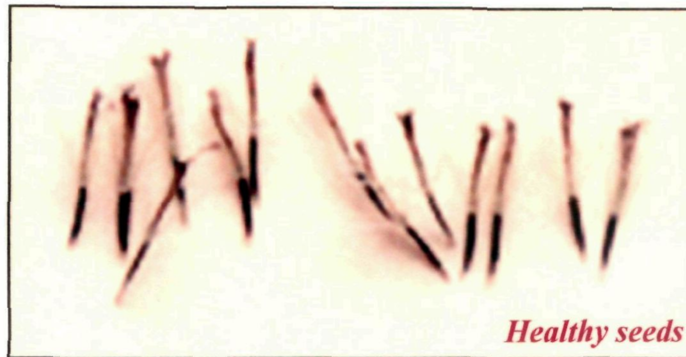
Physiological and biochemical changes due to pathogen-weed interaction in eupatorium was studied in randomly selected plants in each treatment at 7, 14 and 21 days after spray inoculation of the pathogens under field conditions as explained in 'Material and Methods'. The results are presented here under.

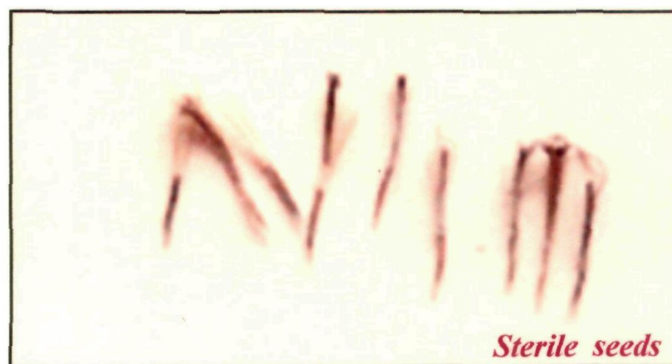
4.12.1 Total chlorophyll content (mg/g fresh wt.)

The data presented in the Table 24 revealed that all the treatments reduced the total chlorophyll content at all the DAS when compared to the control. Highest chlorophyll content was recorded in control (2.42 mg g^{-1} fr. Wt) on 7th day of spraying. Foliar pathogens had greater influence on chlorophyll reduction than floral pathogen *A. pullulans* (2.08 mg g^{-1} fresh. wt at

Table 23b: Effect of *Aureobasidium pullulans* on seed viability of *Chromolaena odorata*

Sl.No.	No. of cypsella / flower	Germinated healthy seeds	Malformed seeds	Sterile seeds
1	30	0	0	30
2	35	2	3	30
3	32	0	30	2
4	34	0	4	34
5	28	0	10	18


Healthy seeds


Sterile seeds

Malformed seeds

Plate 22 : Effect of *Aureobasidium pullulans* on seed viability

(Magnified three times)

Table 24. Effect of foliar spray of pathogens on chlorophyll content and relative water content in *Chromolaena odorata*

Treatments	Total Chlorophyll content (mg/g fresh weight)			Relative water content (%)		
	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS
T1 = *C. gloeosporioides*	2.06	1.80	1.61	82.20	79.80	77.10
T2 = *A. alternata*	2.15	1.86	1.70	83.10	80.00	76.10
T3 = *A. pullulans**	2.26	2.10	2.08	84.20	83.20	80.30
T4 = *A. pullulans* + A. alternata	2.02	1.80	1.66	81.30	79.80	75.20
T5 = *A pullulans* + *C. gloeosporioides*	2.00	1.76	1.51	81.10	78.90	74.30
T6 = *A. alternata* + *C. gloeosporioides*	1.83	1.42	1.04	76.50	70.60	51.90
T7 = *A. pullulans* + *A. alternata* + *C. gloeosporioides*	1.83	1.39	1.03	75.00	66.00	50.50
Control (Water + 3% sucrose)	2.42	2.31	2.13	86.30	85.90	84.20
S.Em±	0.006	0.01	0.01	0.38	0.36	0.47
CD at 5%	0.02	0.04	0.03	1.23	1.02	1.41

DAS = Days after spray inoculation
* = sprayed on flowers

21 DAS). Among individual treatments, *C. gloeosporioides* was significantly superior (1.61 mg g^{-1} fresh wt.) to *A, alternata* (1.70 mg g^{-1} fresh. wt.) at 21 DAS in reducing total chlorophyll. However, combination treatments were more significant to decrease the total chlorophyll content compared to individual treatments. But, *A. pullulans* in combination either with *A. alternata* or *C. gloeosporioides* contributed very less to reduce the total chlorophyll to the extent of 1.66 mg g^{-1} fresh wt. and 1.51 mg g^{-1} fresh wt at 21 DAS respectively.

Maximum reduction in total chlorophyll content was recorded in T_7 (*A. alternata* + *C. gloeosporioides* + *A. pullulans*) with 1.03 mg g^{-1} fresh. wt. which was significantly on par to T_6 (*A. alternata* + *C. gloeosporioides*) with 1.04 mg g^{-1} fresh. wt. at 21 DAS.

4.12.2 Relative water content (RWC) (%)

The results presented in the table 24 indicated that, pathogens had significant influence in decreasing RWC of eupatorium. Maximum RWC was recorded in control (86.3%) at 7 DAS. Decrease in RWC was more in combination treatments than in individual treatments. *A. pullulans* when combined either with *A. alternata* or *C. gloeosporioides* for foliar spraying, contributed very little in decreasing RWC at 21 DAS, i.e., 76.1% to 75.2% and 77.1% to 74.3% respectively. Reduction of RWC was maximum (50.50%) in T_7 (*A. alternata* + *C. gloeosporioides* + *A. pullulans*) followed by T_6 (51.9%) i.e., *A. alternata* + *C. gloeosporioides* at 21 DAS. However, all the treatments were significantly superior to control.

4.12.3 Dry matter accumulation (g/plant) and size of assimilatory surface area (cm^2/plant)

The data on dry matter accumulation and size of assimilatory surface area is presented in the Table 25.

Dry matter accumulation was maximum in the control (16.39 g/plant) at 21 DAS. Infection of leaves/flowers by pathogens, decreased dry matter accumulation significantly at 7, 14 and 21 DAS. However infection of *A. pullulans* on flowers, increased dry matter accumulation (13.96 g/plant) on 14th day after spraying and it was drastically decreased (9.58 g/plant) on 21st day after spraying. Dry matter accumulation was least (7.00 g/plant) in T_7 (i.e. *A. alternata* + *C. gloeosporioides* + *A. pullulans*) followed by T_6 (*A. alternata* + *C. gloeosporioides*) with 7.12 g/plant at 21 DAS.

Size of assimilatory surface area was maximum in the control (1863 cm^2/plant) at 21 DAS. Highest reduction in size of assimilatory surface was recorded in T_7 (*A, alternata* + *C, gloeosporioides* + *A. pullulans*) with 1122 cm^2/plant, followed by T_6 (*A. alternata* + *C. gloeosporioides*) with 1131 cm^2/plant.

4.12.4 Specific leaf weight (mg/cm^2)

Specific leaf weight decreased significantly due to application of pathogens individually and in combinations. The effect was more at later stages (21 DAS) (Table 26). Specific leaf weight was least (5.96 mg/cm^2) when all the pathogens were combined and sprayed i.e., *A. alternata* + *C. gloeosporioides* + *A. pullulans* followed by T_6 (*A. alternata* + *C. gloeosporioides*) with 6.04 mg/cm^2 at 21 DAS. Specific leaf weight was maximum in control at 14 DAS (9.82 mg/cm^2). Spraying of *A, pullulans*) to

Table 25. Effect of foliar spray of pathogens on dry matter accumulation in leaves and size of assimilatory surface area in *Chromolaena odorata*

Treatments	Leaf Dry Weight (g/plant)			Size of assimilatory (cm2/plant)		
	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS
T1 = *C. gloeosporioides*	13.75	12.61	10.93	1573	1510	1473
T2 = *A. alternata*	13.85	12.75	11.13	1575	1524	1482
T3 = *A. pullulans**	12.92	13.96	9.58	1695	1692	1628
T4 = *A. pullulans* + A. alternata	13.55	12.22	9.22	1560	1416	1263
T5 = *A pullulans* + *C. gloeosporioides*	13.51	12.10	9.16	1513	1392	1277
T6 = *A. alternata* + *C. gloeosporioides*	13.45	10.82	7.12	1394	1318	1131
T7 = *A. pullulans* + *A. alternata* + *C. gloeosporioides*	13.33	10.51	7.00	1388	1316	1122
Control (Water + 3% sucrose)	14.16	15.51	16.39	1776	1811	1863
S.Em±	0.05	0.18	0.10	4.0	4.5	5.1
CD at 5%	0.18	0.53	0.32	11.6	13.2	14.6

DAS = Days after spray inoculation
* = Sprayed on flowers

Table 26. Effect of spraying of pathogens on specific leaf weight (mg/cm2) in *Chromolaena odorata*

Treatments	7DAS	14DAS	21DAS
T1 = *Colletotrichum gloeosporioides*	8.63	7.83	7.42
T2 = *Alternaria Alternata*	8.79	7.83	7.51
T3 = *Aureobasidium pullulans**	8.10	8.99	7.03
T4 = *A. pullulans* + *A. alternata*	8.61	7.61	7.30
T5 = *A. pullulans* +*C. gloeosporioides*	8.53	7.53	7.17
T6 = *A. alternata* + *C. gloeosporioides*	8.41	6.71	6.04
T7 = *A. pullulans* + *A. alternata* + *C. gloeosporioides*	8.39	6.66	5.96
Control (Water + 3% sucrose)	9.61	9.82	9.23
S.Em±	0.08	0.10	0.12
CD at 5%	0.23	0.31	0.29

DAS = Days after spraying

* = Sprayed on flowers

flowers slightly increased specific leaf weight (8.99 mg/cm^2) at 14 DAS, which decreased greatly (7.03 mg/cm^2) at 21 DAS.

4.12.5 Total sugars (mg/g dry weight)

The results revealed that, all treatments were effective in reducing the total sugar content in the plants (Table 27). Highest sugar content was recorded (23.8 mg/g dry wt.) in control at 14 DAS. Generally, total sugar content was decreased along with the incubation period i.e., 7, 14 and 21 DAS. Total sugar content was least (11.8 mg/g dry wt.) in T_7 (*A. alternata* + *C. gloeosporioides* + *A. pullulans*) followed by T_6, *A. alternata* + *C. gloeosporioides* with 12.1 mg/g dry wt. at 21 DAS. Generally combination of treatments were significantly superior over individual treatments in reducing the sugar content. *C. gloeosporioides* reduced the total sugar content to maximum extent (16.8 mg/g dry wt.) followed by *A. alternata* (17.9 mg/g dry wt.) and *A. pullulans* (18.3 mg/g dry wt) at 21 DAS.

4.12.6 Tannin content (mg/g dry wt.)

The results presented in the Table 27 indicated that, all the treatments significantly decreased the tannin content compared to the control. Lowest tannin content was recorded in T_7 (4.33 mg/g dry weight) followed by T_6 (4.92 mg/g dry wt.) at 21 DAS. Highest tannin content was recorded in control (11.82 mg/g dry weight) at 7 DAS.

4.12.7 Nitrate reductase activity (NRA) μ mole NO_2 g fresh wt. min^{-1}

The data presented in the Table 28 revealed that, the NRA was decreased in all the treatments at all the DAS tested. Maximum NRA was recorded in the control (4.23 μ mole NO_2 g fresh wt. min^{-1}) at 7 DAS. Decline in

Table 27. Effect of foliar spray of pathogens on total sugar and tannin content in *Chromolaena odorata*

Treatments	Total sugars (mg/g dry weight)			Tannin content (mg/g dry weight)		
	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS
T1 = *C. gloeosporioides*	19.2	18.7	16.8	9.83	8.10	6.91
T2 = *A. alternata*	19.8	19.6	17.9	10.02	8.30	7.23
T3 = *A. pullulans**	20.3	20.1	18.3	10.31	9.60	8.16
T4 = *A. pullulans* + A. alternata	18.3	17.5	16.6	9.61	7.51	6.32
T5 = *A pullulans* + *C. gloeosporioides*	18.1	16.3	15.5	9.44	7.03	5.31
T6 = *A. alternata* + *C. gloeosporioides*	17.6	16.1	12.1	9.41	6.51	4.92
T7 = *A. pullulans* + *A. altemata* + *C. gloeosporioides*	17.4	15.5	11.8	9.22	5.53	4.33
Control (Water + 3% sucrose)	23.4	23.8	23.5	11.82	10.91	10.23
S.Em±	0.51	0.35	0.33	0.31	0.41	0.55
CD at 5%	1.21	1.03	0.98	0.81	1.23	1.69

DAS = Days after spray inoculation
* : Sprayed on flowers

NRA level was more at later stages of infection i.e. at 21 DAS. Least NRA (1.53 μ mole NO_2 g fresh wt. min^{-1}) was recorded in T_7 treatments i.e. *A. alternata* + *C. gloeosporioides* + *A. pullulans* followed by T_6 (1.58 μ mole NO_2 g fresh wt. min^{-1}) i.e. *A. alternata* + *C. gloeosporioides* at 21 DAS. Among individual treatments *C. gloeosporioides* reduced NRA (2.95 μ mole NO_2 g fresh wt. min^{-1}) to maximum extent compared to the other individual treatments i.e. *A. alternata* (3.03 μ mole NO_2 g fresh wt. min^{-1}) and *A. pullulans* (3.16 μ mole NO_2 g fresh wt. min^{-1}) at 21 DAS.

4.12.8 Total free phenols (mg/g dry weight)

In general, infection due to pathogen led to accumulation of total free phenols in the infected tissues and the effect was more at the early stages (Table 28) of infection. Accumulation of total free phenols was maximum (14.21 mg/g dry wt.) in T_7 *A. alternata* + *C. gloeosporioides* + *A. pullulans* at 21 DAS which was significantly on par with T_6 treatment (14.03 mg/g dry wt.) i.e. *A. alternata* + *C. gloeosporioides* at 21 DAS. In contrast, total free phenol content in control was ranged from 8.83 to 11.41 mg/g dry weight at 7 DAS and 21 DAS, respectively.

4.12.9 Free amino acids (mg/g dry wt.)

From the table 28, it is clear that, all the treatments were superior to decrease the amount of free amino acids. Amino acids were decreased to maximum extent in T_7 treatment (1.51 mg/g dry wt.) i.e. *A. alternata* + *C. gloeosporioides* + *A. pullulans* which was significantly on par with T_6 (1.82 mg/g dry wt.) i.e. *A. alternata* + *C. gloeosporioides* at 21 DAS. Maximum content of free amino acids was recorded in control with 4.61 mg/g dry wt. at 7 DAS.

Table 28. Effect of foliar spray of pathogens on nitrate reductase activity, total free phenols and amino acid content in *Chromolaena odorata*

Treatments	Nitrate reductase activity (μ NO_2 g fresh wt.min^{-1})			Total free phenols (mg/g dry weight)			Free amino acids (mg/g dry weight)		
	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS
T1 =*C. gloeosporioides*	3.93	3.41	2.95	9.82	11.93	12.98	4.01	3.62	3.01
T2 = *A. alternata*	4.10	3.61	3.03	9.63	11.41	12.12	4.16	3.71	3.23
T3 = *A. pullulans**	4.16	3.80	3.16	9.16	11.2	12.03	4.23	3.96	3.51
T4 = *A. pullulans* + *A. alternata*	3.72	3.23	2.81	9.93	12.10	12.92	3.92	3.22	2.91
T5 = *A pullulans* + *C. gloeosporioides*	3.70	3.20	2.71	10.12	12.40	13.05	3.81	3.16	2.62
T6 = *A. alternata* + *C. gloeosporioides*	3.60	2.91	1.58	10.60	12.70	14.03	3.73	2.95	1.82
T7 = *A. pullulans* + *A. alternata* + *C. gloeosporioides*	3.40	2.72	1.53	10.91	12.91	14.21	3.41	2.77	1.51
Control (Water + 3% sucrose)	4.23	4.16	3.93	8.83	10.31	11.49	4.61	4.23	4.11
S.Em±	0.16	0.16	0.18	0.50	0.59	0.40	0.19	0.31	0.24
CD at 5%	0.51	0.46	0.56	1.51	1.62	1.23	0.56	0.82	0.73

DAS =Days after spray inoculation
* = Sprayed on flowers

4.12.10 Photosynthetic rate (μ mole $CO_2/dm^2/s$)

Spraying of foliar as well as floral pathogens significantly reduced the photosynthetic rate (Table 29). Photosynthetic rate was found to be decreased at all the stages in all the treatments except control. Photosynthetic rate was highest in the control (12.9 μ mole/$CO_2/dm^2/s$) at 7 DAS. It was least in T_7 (3.00 μ moler CO_2 /dm^2/s) i.e., *A. alternata* + *C. gloeosporioides* + *A. pullulans* combination which was significantly on par with T_6 (*A. alternata* + *C. gloeosporioides)* with 3.15 μ mole $CO_2/dm^2/S$ at 21 DAS. Combination treatments were more superior to individual treatments in decreasing photosynthetic rate. Among the individual treatments, spraying of *A. pullulans* on flowers slightly increased the photosynthetic rate (11.26 μ mole $CO_2/dm^2/s$) at 14 DAS and which sharply declined (5.15 μ mole $Co_2/dm^2/s$) at 21 DAS. Reduction in photosynthetic rate was on par in T_4 i.e. *A. alternata* + *A. pullulans* (6.40 μ mole $CO_2/dm^2/s$) with T_5 i.e., *A. pullulans* + *C. gloeosporioides* (6.00 μ mole $CO_2/dm^2/s$).

4.12.11 Transpiration rate (μ g H_2o m^{-2} s^{-1})

Transpiration rate was significantly decreased in all the treatments with greater effect at later stages (Table 29). Maximum transpiration (11.81 μ g H_2O m^{-2} 5^{-1}) was recorded in control at 7 DAS. Reduction in transpiration rate was maximum (3.15 μ g H_2O m^{-2} 5^{-1}) in T_7, significantly on par to T_6 (*A. alternata* + *C. gloeosporioides*) with 3.45 μ g H_2O m^{-2} 5^{-1} at 21 DAS. Among individual treatments *A. pullulans* reduced the transpiration rate (4.51 μ g H_2O m^{-2} 5^{-1}) to the maximum extent at 21 DAS.

Table 29. Effect of foliar spray of pathogens on photosynthetic rate, transpiration rate and leaf temperature in *Chromolaena odorata*

Treatments	Photosynthetic rate (μ mole $CO_2/dm^2/s$)			Transpiration rate (μ g H_2O m^{-2} s-1)			Leaf temperature (°C)		
	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS	7DAS	14DAS	21DAS
T1 = *C. gloeosporioides*	10.16	8.61	6.17	8.82	7.22	5.12	31.20	31.30	31.50
T2 = *A. Alternata*	10.31	8.83	6.92	9.13	7.91	5.93	30.90	31.20	31.40
T3 = *A. pullulans**	10.65	11.26	5.15	8.10	9.62	4.51	30.80	30.90	31.20
T4 = *A. pullulans* + *A. alternata*	9.83	7.33	6.50	8.41	6.92	4.90	31.00	31.50	31.80
T5 = *A. pullulans* + *C. gloeosporioides*	9.10	7.12	6.00	8.03	6.51	4.66	31.70	31.80	32.10
T6 = *A. alternata* + *C. gloeosporioides*	8.00	6.31	3.15	7.93	6.13	3.45	31.90	32.10	32.40
T7 = *A. pullulans* + *A. alternata* + *C. gloeosporioides*	7.91	6.62	3.00	7.91	5.83	3.15	32.00	32.30	32.80
Control (Water + 3% sucrose)	12.9	12.2	11.93	11.81	10.62	9.83	30.30	30.60	30.10
S.Em±	0.13	0.18	0.13	0.21	0.23	0.19	0.58	0.50	0.47
CD at 5%	0.42	0.53	0.40	0.63	0.72	0.56	1.76	1.51	1.43

DAS =Days after spray inoculation
* = Sprayed on flowers

4.12.12 Leaf temperature (°C)

The results are presented in the Table 29. It was noticed that there was slight increase in leaf temperature when pathogens were sprayed on foliage and flowers. Leaf temperature ranged from 30.10°C (control) to 32.80°C (*A. alternata* + *A. pullulans* + *C. gloeosporioides*) followed by 32.40°C in *A. alternata* + *C. gloeosporioides* combination at 21 DAS.

DISCUSSION

V. DISCUSSION

Chromolaena odorata King and Robinson (=*Eupatorium odoratum* L.) is an alien, noxious perennial woody shrub. It is a serious weed in wild life sanctuaries and plantations of cocoa, cashew, cardamom, arecanut, coconut, tea, coffee and citrus. It is a menace in young, establishing forests and forest nurseries. It has been adopted in the evolutionary process to grow and multiply and perpetuate effectively under low light intensity areas apart from its existence in open lands. It smothers native vegetation, young trees and plantations due to its competitive nature owed to aggressive vegetative growth. Due to its allelopathic potentiality, it leads to poor crop establishment. It has become a serious weed both in lower and higher altitudes of the North-eastern regions, Western Ghats and Nilgiris, which are the bio-diversity rich areas of India (Bunnett and Rao, 1968; Muniappan and Viraktamath, 1993).

Attempts to control the weed by mechanical and chemical methods are not economical and feasible approaches. Only biological suppression using pathogens or insects will provide the long-term solution in the management of the weed economically on sustainable basis. Biological control attempts using *Paredchaetes pseudoinsulata, Apion brunne onigrum* and *Mescinia parvula* were not successful as they failed to establish under field conditions. By looking to the serious threat posed by eupatorium and dearth of technology in the management aspect of the weed, present investigation was initiated with the following objectives.

1) Collection and isolation of different pathogens associated with the weed and selection of potential pathogens.

2) Host specificity study, mass production and field efficacy of the pathogens in weed management.

The plant parts exhibiting disease symptoms were collected from different localities in the districts of Belgaum, Dharwad, Uttara Kannada and Shimoga. The pathogens associated with *C. odorata* were isolated following tissue isolation procedure. The pathogen isolated from each locality was considered as an isolate and thus eighteen isolates were identified with the help of Agharkar Research Institute, MACS, Pune (Maharashtra). Isolated pathogens were maintained in pure culture on potato dextrose agar.

Barreto and Evans (1994) carried out two year survey and identified several fungal species associated with *C. odorata* in Southern Brazil.In the present study, all the eighteen isolates were found to be fungal pathogens, distributed in nine different genera. Among them, *Alternaria alternata* appeared to be a common pathogen that was recorded from eight different localities *viz.,* Belgaum, Khanapur, Kalaghatagi, Sirsi, Yellapur, Hubli, Shimoga and Hosanagar. *A. alternata* caused a typical target board symptom mostly on older leaves, leaf necrosis, wilting and premature defoliation. The pathogen has not been recorded previously from *C. odorata*, although, it appears to be a cosmopolitan fungus with a wide host range. And hence, this is the first report of *A. alternata* on *C. odorata*. Barreto and Evans (1994) reported, *A. zinniae* on *C. odorata* from Southern Brazil.

Aureobasidium pullulans was collected from only one site, Mundagod and it caused severe black mould on inflorescence of eupatorium. There is no earlier record of *A. pullulans* on *C. odorata* either from India or elsewhere and hence, it is a new report.

Beltrania rhombica was collected from only one locality *viz.*, Sagar of Shimoga district which caused leaf spots. This is the first record of *B. rhombica* on eupatorium weed.

The other new species, *Bipolaris* sp was collected from two different localities viz Sagar and Londa, which caused leafspots. This is a new record of *Bipolaris* sp on *C. odorata.*

Colletotrichum gloeosporioides was recorded from two different locations of Dharwad district viz., Prabhunagar and Dharwad. During survey, it was noticed that, *C. gloeosporioides* appeared more on younger leaves than on older ones and resulted in shot holes, drying, curling symptoms leading to premature defoliation. Until so far, there is no report of *C. gloeosporioides* on *C. odorata* and hence this is the first report from India. However, Litzenberger *et al* (1963) reported *Colletotrichum* sp on *C. odorata* from Colombia.

During survey, *Phyllosticta* sp. was recorded from only one location Haliyal which caused leaf spots. Earlier workers (Viegas, 1961; Yen, 1979) reported *Phyllosticta eupatoriicola* Yen on *C. odorata*. *Phoma eupatorii* was isolated from only one site, Mundagod which caused leafspots. The fungus was earlier reported from India (Pers. Comm) on eupatorium species. The *Phomopsis* sp caused leaf spots on eupatorium, which was collected from Sirsi. Similar observation was recorded from Srilanka (Barreto and Evans, 1994).

Thus, there were eighteen pathogenic isolates of eupatorium, distributed in nine different genera, viz., *Alternaria*, *Aureobasidium*, *Beltrania*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Phyllosticta*, *Phoma* and *Phomopsis*.

All the isolates satisfied the Koch's postulates and hence proved to be pathogenic to the weed. However, they varied in their ability to cause the disease severity on eupatorium.

Bioherbicidal strategy will be more sound only when highly virulent isolates were used. Selection of highly virulent isolates to induce maximum

disease on the weed was carried out under glasshouse conditions. Among *A. alternata* isolates, Yellapur isolate was the most virulent of all the isolates under study. It caused the necrosis to the maximum extent (80.00 PDI). *A. pullulans* caused cent per cent black mould on inflorescence however, it induced a few minute lesions on leaves also (Table 2).

Preliminary screening of two isolates of *Colletotrichum gloeosporioides* indicated the equipotentiality of both the isolates to cause disease on the weed. As they were on par with respect to virulence, both were selected for further screening. *Phoma eupatorii*, *Phyllosticta* sp. and *Phomopsis* sp. were found to be moderately virulent to cause leaf spot on the weed.

Other pathogens viz., *Bipolaris* sp., *Beltrania rhombica* and *Cladosporium spherospermum* were least virulent. Though, they caused leaf spots they failed to increase the disease severity further along with incubation period. As they were not found as promising candidates for biocontrol of weeds, they were deleted from the present studies. Gayathri and Pandey (1997) screened and selected only virulent isolates of *C.gloeosporioides* to control Parthenium, based on per cent disease index and also lesion type.

Based on the results of *in vivo* screening of eighteen isolates for their virulence, only seven isolates were selected viz., *Alternaria alternata* (Yellapur isolate), *Colletotrichum gloeosporioides* (Prabhunagar and Dharwad isolates), *Aureobasidium pullulans* (Mundagod), *Phyllosticta* sp.(Haliyal),*Phoma eupatorii* (Mundagod) and *Phomopsis* sp.(Sirsi). They were found moderately virulent to virulent and hence they were included in the second phase of screening studies. In this step, seven different pathogenic isolates were screened and selected based on their potentiality to grow and sporulate on different media.

Cultural and morphological studies were carried out to find out the best medium for the growth and sporulation of isolates. The medium, which supported maximum growth and sporulation, was selected as best medium. The isolates, which exhibited maximum growth and sporulation, were considered as 'Potential pathogens'.

Cultural characteristic studies on different solid media showed the variation among seven pathogenic isolates of *C. odorata* with respect to colony characters like colour of the colony, type of growth and colony margin. (Barreto and Evans, 1994). In the present study, isolates of *A alternata* (Yellapur isolate), *C. gloeosporioides* (Dharwad and Prabhunagar isolates), *A. pullulans* (Mundagod), *Phomopsis* sp. (Sirsi), *Phyllosticta* sp. (Haliyal) exhibited light dark black coloured colony, except *Phoma eupatorii* which produced red coloured colony. Isolates *Phyllosticta* sp., *Phomopsis* and *A. pullulans* exhibited elevated to submerged growth and serrated to smooth margin on PDA medium.

The pathogenic isolates varied in their ability to grow and sporulate on different media. Among the seven isolates, *A. pullulans* showed highest growth (61.67 mm) and excellent sporulation (3.84). Among two isolates of *C. gloeosporioides,* Prabhunagar isolate was more efficient in its growth (58.33 mm) and sporulation capacity (3.50) compared to Dharwad isolate (50.88 mm and 2.66) and hence, *C. gloeosporioides* of Prabhunagar was selected in the present study. Eventhough *A. alternata* (Yellapur) recorded least growth (35.78 mm), it sporulated fairly (2.22) and it was able to cause disease significantly (Table 2).

Though, isolates *Phoma eupatorii*, *Phomopsis* sp. and *Phyllosticta* sp showed good growth they sporulated poorly. Mycoherbicidal tactic to be successful it must be possible to produce abundant spores in artificial culture

(Daniel *et al.,* 1973; Charudattan, 1991). As these three isolates did not fulfill one of the attributes of mycoherbicide, these were rejected from the further study.

The results of this experiment indicated that, only three pathogenic isolates *viz.*, *A. alternata* (Yellapur), *C. gloeosporioides* (Prabhunagar isolate) and *A. pullulans* (Mundagod) satisfied the features of mycoherbicides and hence they were tagged as 'Potential Pathogens' of *C. odorata*.

Among the several media screened, PDA supported maximum growth (55.00 mm) followed by Sabouraud's dextrose agar (48.85 mm) and Richards's agar (44.66 mm). Sporulation was supported to the maximum extent in PDA (2.57) followed by Host extract + 1per cent sucrose (2.28), Host extract agar (2.14) and Richards's agar (2.14). Tochinai's agar and oatmeal agar failed to support good growth and sporulation of the pathogens respectively.

Growth phase study of three potential pathogens revealed that, the maximum growth of *A. alternata* was on the 12th day of seeding, where as, *C. gloeosporioides* and *A. pullulans* required 10 days of incubation period for the maximum growth. Later dry mycelial weight of the pathogens decreased, with the increase in the incubation period. This may be due to autolysis of the mycelium and exhaustion of nutrients in the medium. Lilly and Barnett (1951) discussed that the growth in fungi follows a definite pattern and they observed the onset of autolysis after the maximum growth during which cellular enzymes begin to digest the various cell constituents. Hence for other studies, harvesting was made on the 12th day for *A. alternata* and on the 10th day for *A. pullulans and* C. *gloeosporioides.*

Fungi possess an ability to utilize a wide range of nutrients and in the radial measurement it is not possible to consider the amount of submerged mycelium. Hence, Cochrane (1958) has opined the determination of dry

mycelial weight as the best method for precise research work. In the present study, among the liquid media tested, PDB supported maximum growth of all the pathogens. Next best media for *A. alternata* were Richards's medium followed by Sabouraud's dextrose broth and oatmeal medium (Table 8). The ability of the fungus to grow more on non-synthetic media indicated the requirement of nutrients present in that medium for growth of the pathogens. *A. pullulans* and *C. gloeosporioides* recorded excellent growth on PDB followed by Sabouraud's dextrose broth. Among the synthetic media tested, Richards's medium supported good growth of the fungi and hence used as a basal medium wherever synthetic medium is required. Otherwise, semi-synthetic medium Sabouraud's dextrose broth was used in physiological, nutritional and biochemical studies.

Temperature is an important factor governing distribution, growth, reproduction and survival of the fungus. All fungi have minimum temperature below, which they cannot grow and above which they are inactivated. Each fungus has its temperature range for growth and sporulation. In the present study the temperature range of 25 to 30 °C was found to be optimum for the growth of pathogens (Guo *et al.,* 1992). Togashi (1949) stated that a number of plant pathogenic fungi have optima in the range of 20-30 ° C and about half of them have their optimum range between 26 to 30°C. It is evident that incubation at 30°C favoured maximum growth of *Alternaria alternata* and *Aureobasidium pullulans*, where as 25°C supported maximum growth of *Colletotrichum gloeosporioides*. Hildebrand and Jensen (1991) reported that optimum temperature for mycelial growth of *C. gloeosporioides*, a bioherbicide of St. John's-wort weed, on agar medium was 24°C.

Growth of all the three pathogens was found ceased at 45°C. However, moderate growth of *A. alternata* and *A. pullulans* was obtained in the range of

20 – 35°C, whereas moderate growth of *C. gloeosporioides* was recorded in the range of 20-30°C. All the pathogens showed poor growth at 40°C.

The pathogens' preference for the pH levels varied considerably. In the present study, it was noticed that, *A. alternata* and *C. gloeosporioides* preferred acidic to neutral pH. There was sudden decline in the growth from pH 7.00 onwards (Table 10). This rate of decline in mycelial growth on alkaline side of neutrality indicated that, fungus was acid tolerant. Cochrane (1958) and Bilgrami and Verma (1978) also opined that in contrast to bacteria and actinomycetes fungi are relatively most tolerant of acid ions (H^+) than basic ions (OH^-). Optimum pH for *A. alternata* was pH 5.50. Similar results were reported by Deshpande *et al.* (1997) for the growth of *A. alternata*, a bioherbicide of parthenium. Maximum growth of *C. gloeosporioides* was favoured by pH 6.50 and growth was nil at pH 8.0 and 8.50.

On the contrary, *A. pullulans* was capable of growing at wide pH range (4.00 to 8.50). However, it showed peak growth at different pH levels., i.e. 6.00 and 8.00. It exhibited moderate growth at pH levels 5.50, 6.50, 7.00 and 7.50. This type of behaviour of *A. pullulans* might be attributed to its dimosphic nature (Park, 1984). *A. pullulans* produces blastospores, swollen cells and chlamydospores which are influenced by different pH levels and transition of one phase to other is influenced by pH levels (Bermejo *et al.*, 1981; Park, 1984).

Carbon is the most essential element required by fungi, since it comprises of about 50 per cent of total mycelial weight as a component of both structural and functional constituents (Bilgrami and Verma, 1978). The amount and type of sugar in media or in the host may affect the growth of pathogens considerably. In the present study, dextrose was found to be the best source

of carbon for growth of all the three pathogens. Next best carbon source was sucrose for the growth of *A. alternata* and *C. gloeosporioides*, whereas *A. pullulans* equally preferred glucose and sucrose. This might be due to ability of the pathogens to preferentially utilize carbon from these sources. Dextrose being the major component of photosynthetic plants is generally utilized as a good carbon source by most of the plant pathogenic fungi (Lilly and Barnett, 1951). Lactose failed to support the good growth of *C. gloeosporioides*. Lactose can be considered as an uncommon sugar for the plant pathogenic fungi, since they are not likely to encounter with this sugar in nature. However, pathogens *A. alternata* and *A. pullulans* showed growth with lactose as carbon source. Galactosidase an enzyme responsible for breaking down lactose into galactose and glucose has not been commonly reported in fungi, but adaptive enzyme system which enable them to grow in such uncommon sugars has been discussed (Cochrane, 1958). Both *A. alternata* and *A. pullulans* exhibited poor growth in citric acid, as it was not preferred for the growth. Organic acids like citrate and tartrate favour the growth of fungi and only under restricted conditions (Cochrane, 1958).

Nitrogen is very important element for protein synthesis. There is considerable diversity in the ability of fungi to utilize various nitrogen sources. In the present study, peptone was the most preferred nitrogen source for the growth of all the pathogens. Cochrane (1958) opined that peptone, a mixture of peptides of varying chain length is generally easily utilized and also serve as an adequate source of nitrogen for many fungi. Similar results were reported by Guo *et al.* (1992) in *Mycovellosiella eupatorii odorai* against *C. odorata*.

A. alternata showed best growth in potassium nitrate followed by ammonium nitrate. According to Lilly and Barnett (1951), nitrates are better utilized by fungi both for the purpose of growth and reproduction.

Maximum mycelial growth of *C. gloeosporioides* was observed in peptone followed by tyrosine and asparagine. The poor growth of *C. gloeosporioides* was observed in methionine and urea. Cochrane (1958) has opined that, breakdown of urea to ammonia on autoclaving and ammonia inturn in high concentration is toxic to fungi.

According to Lilly and Barnett (1951) with the exception of certain aminoacids (Primary amino acids) which enter directly into metabolic pathway leading to the synthesis of protein. Most nitrogen sources undergo modification before entering the synthetic metabolic pathway. Further, they are of the opinion that these (secondary amino acids) amino acids which do not enter directly into metabolic pathway are probably broken down and then used for protein synthesis. This may be the reason for poor growth of the fungus in aminoacids like methionine.

Good growth of pathogen in these aminoacids may indicate the direct utilization of these aminoacids in protein synthesis (Lilly and Barnett, 1951). In the present study, *A. pullulans* favoured peptone followed by potassium nitrate and ammonium nitrate (Ramos and Garcia, 1975; Bermejo *et al.*, 1981 ; Park, 1982).

One of the recent approaches in weed management is exploitation of secondary metabolites of fungi as phytotoxins. In the present study, effect of culture filtrate of potential pathogens *viz., Alternaria alternata, Aureobasidium puliulans* and *Colletotrichum gloeosporioides* was assayed on eupatorium and tomato cuttings. The results revealed that, crude filtrate of *A. alternata* induced necrosis, drooping and wilting of cuttings of tomato and eupatorium after 24 h of incubation. Similar symptoms were noticed on eupatorium stem cuttings dipped in 1:1 dilution after 36 h of incubation.

A. pullulans induced drooping, curling, brittling and wilting within 24 h of incubation. Whereas, in 1:1 diluted culture filtrate, symptoms were noticed after 32 h of incubation.

Necrosis, brittling and wilting of leaves in metabolites indicate the presence of phytotoxins in the culture filtrate (Yang *et al.*, 1990). *A. alternata* and *A. pullulans* were capable of producing toxins, whereas, *C. gloeosporioides* could not produce any toxins against the weed and tomato cuttings.

A critical consideration in the development of a biological agent for weed control is the determination of host range. Irrespective of potential benefits, the safety of nontarget cultivated plants and wild plants must be ensured prior to use. With endemic pathogens used as inundative inoculum, or bioherbicides, increased disease pressure on cultivated plants or potential conflicts of interest issues must be avoided.

In the present study, host range of potential pathogens was tested on weed as well as on cultivated plants. It was observed that, potential pathogens were incapable of infecting the weed plants i.e. *Parthenium* and *Lantana camera*. Eventhough, *A. alternata*, produced mild lesions on *L. camera*, microscopic observations indicated that, the pathogen failed to ramify in the host tissues. Plantation crops *viz.*, arecanut, coconut, pepper, betelvine, cardamom, cocoa, coffee, banana and cashew were free from infection and remained healthy. This indicated that, the potential pathogen were safe to use against eupatorium which is a common menace in plantations. Field crops *viz.*, cotton, paddy, sunflower and cowpea were immune to infection and hence showed no symptoms. None of the pathogens caused symptoms on any of the forest tree tested. Kauraw and Bhan (1993) reported similar results on field crops while working with biocontrol of parthenium with Fusarium

pallidoroseum. The results of the host range studies indicated that, the potential pathogens of *C. odorata, viz., A. alternata, A. pullulans* and *C. gloeosporioides* were host specific and very safe to be used under natural ecosystems without harming any plantation, field and forest crops tested. Hence, they can be used to manage *C. odorata*. The results are in line with the findings of Elango *et al.* (1993) and Barreto and Evans (1994).

Daniel *et al.* (1973) discussed that an endemic pathogen might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particularly susceptible stage of weed growth. Because endemic diseases evolve in regions of host parasite co-adaptation, it is logical to find them in native or naturalized range of weeds (Charudattan, 1988). Hence a second year survey and surveillance was carried out during 1996-97 at vegetative and flowering stages to know whether the potential pathogens selected are endemic or not. The study confirmed that all three potential pathogens associated with *C. odorata* were endemic with their maximum per cent recovery. *A. alternata* was endemic in Yellapur with its characteristic target board effect and veinal necrosis symptoms. *A. pullulans* caused black mould of inflorescence and floral infection was maximum in Mundagod thus confirming the endemic nature of the pathogen. Isolation of diseased samples with leaf spot symptoms collected from Prabhunagar, indicated the endemic nature of *C. gloeosporioides*.

Inoculation is the application of inoculum to the infection court of an intended host plant. Among the different methods of inoculation techniques, spraying method of inoculation induced the disease on eupatorium and other techniques viz., seed, soil and stem inoculation techniques were futile to induce any symptoms on the weed. As *A. alternata* and *C. gloeosporioides* were inhabitants on foliage of *C. odorata*, spray inoculation on foliage caused leaf

spots. *A. pullulans* was noticed on flowers of *C. odorata* and spraying on flowers resulted in black mould and few mild lesions on foliage.

As majority of pathogens associated with the weed were being foliar pathogens, earlier workers followed 'spray inoculation technique' to induce disease on weeds (Walker and Sciumbato, 1979; Morin *et al.*, 1989; Makowski, 1993).

Application of high levels of inoculum of a mycoheribicide at particular susceptible growth stage of a weed may compensate for possible restraints such as suboptimal environmental conditions, low pathogenic virulence or host resistance preventing a disease epidemic (Templeton *et al.*, 1979).

It was noticed that, extent of foliar and floral disease of eupatorium caused by *A. alternata, C. gloeosporioides* and *A. pullulans*, respectively, was dependent on the amount of inoculum sprayed on the plant. And inoculum concentration required to cause maximum disease, varied for each pathogen. In general, increase in inoculum density increased the disease severity but upto certain level.

Per cent disease index was maximum (72.02%) when *A. alternata* was sprayed at a concentration of 10^{10} spores/ml, whereas, *C. gloeosporioides* required a higher spore concentration by 10^{14} spores/ml to cause highest per cent disease index (82.12%). *A. pullulans* caused maximum infection on flowers at spore concentration of 10^{16} spores/ml. All types of spores produced by *A. pullulans* viz., blastospores and conidia were included during total spore counting

Disease was decreased gradually after optimum spore concentration. Further, even though spore concentration was increased, disease level was not

enhanced especially in foliar pathogens. This may be attributed to auto inhibition of spore germination at higher concentrations (Heiny and Templeton, 1991). It is possible that, plant surface may compensate for this growth inhibition, through interaction with the chemical characteristics of the plant cuticle or dilution due to spatial redistribution on plant surface (Hoagland, 1995).

In further studies, standardized spore load of 10^{10} spores/ml, 10^{14} spores/ml and 10^{16} spores/ml for *A. alternata*, *C. gloeosporioides* and *A. pullulans* respectively, were used to create maximum disease pressure on Eupatorium. Plants do have some crucial stage, at which they succumb to stress, may be biotic or abiotic. Identification of crucial growth stage of the weed for maximum disease induction is an essential step in mycoherbicidal approach.

In the present investigation, it was observed that, effectiveness of inoculum densities in killing *C. odorata* was related to plant growth stage. Eupatorium plants at younger stage i.e., two months old were highly susceptible to *C. gloeosporioides* @ 10^{14} spores/ml. Whereas, *A. alternata* favoured three months old plants and older leaves with maximum infection (72.02 per cent disease index) when sprayed @ 10^{10} spores/ml. In general, *C. gloeosporioides* was observed more on younger leaves, whereas *A. alternata* was more on older leaves. This may be attributed to preference of pathogens to sugar at varying levels. *A. alternata* has been classified as low sugar pathogen, where lower sugar helps in the growth of the pathogen (Horsfall and Dimond, 1959) and hence it might have recorded more on older leaves. Where as *C. gloeosporioides*, being a high sugar pathogen, it favoured young plants (Horsfall and Dimond, 1959). Deshpande *et al* (1997) reported that, the

parthenium plants at two leaf stage were highly susceptible to *A. alternata.*

In the present study, it was observed that, *A. pullulans* was a poor pathogen on leaves but, It was highly pathogenic and caused severe infection on inflorescence. However, its impact on seed biology was dependent on inflorescence stage at which it infects. *A. pullulans* could not infect either at bud initiation or flower initiation stage. Its infection on opening flowers resulted in complete destruction of buds, whereas infection at full bloom stage resulted in sterile and malformed seeds. Thus, *A. pullulans* as a floral pathogen had tremendous impact in decreasing seed viability, there by reduced the weed seed bank in the soil. On matured seeds, it had no effect and the infection was only superficial. *A. pullulans* as a floral pathogen might have inhibited floral development, embryo development and seed filling. The weed is capable of producing large quantity of seeds with estimates ranging from 93,000 to 16,00,000 per plant (Wilson, 1995). The rapid spread of the weed is due to extensive seed production and wind dispersal nature of the seeds (Mac donald and Frame, 1988). With this background, the floral pathogen *A.pullulans* appears to be more promising as it destructs the seed production thus reducing seed rain and weed seed bank in the soil. Production of chlamydospores and diamorphic nature of *A. pullulans* may become an added advantage to develop it as a potential mycoherbicide (Plate 23).

Histo-pathological changes observed during infection might be attributed to consumption of nutrients in the host tissue by the pathogen (TeBeest *et al.,* 1978).

Interaction among the potential pathogens and their combined effect on weed management was studied. Interaction effect was analysed based on the

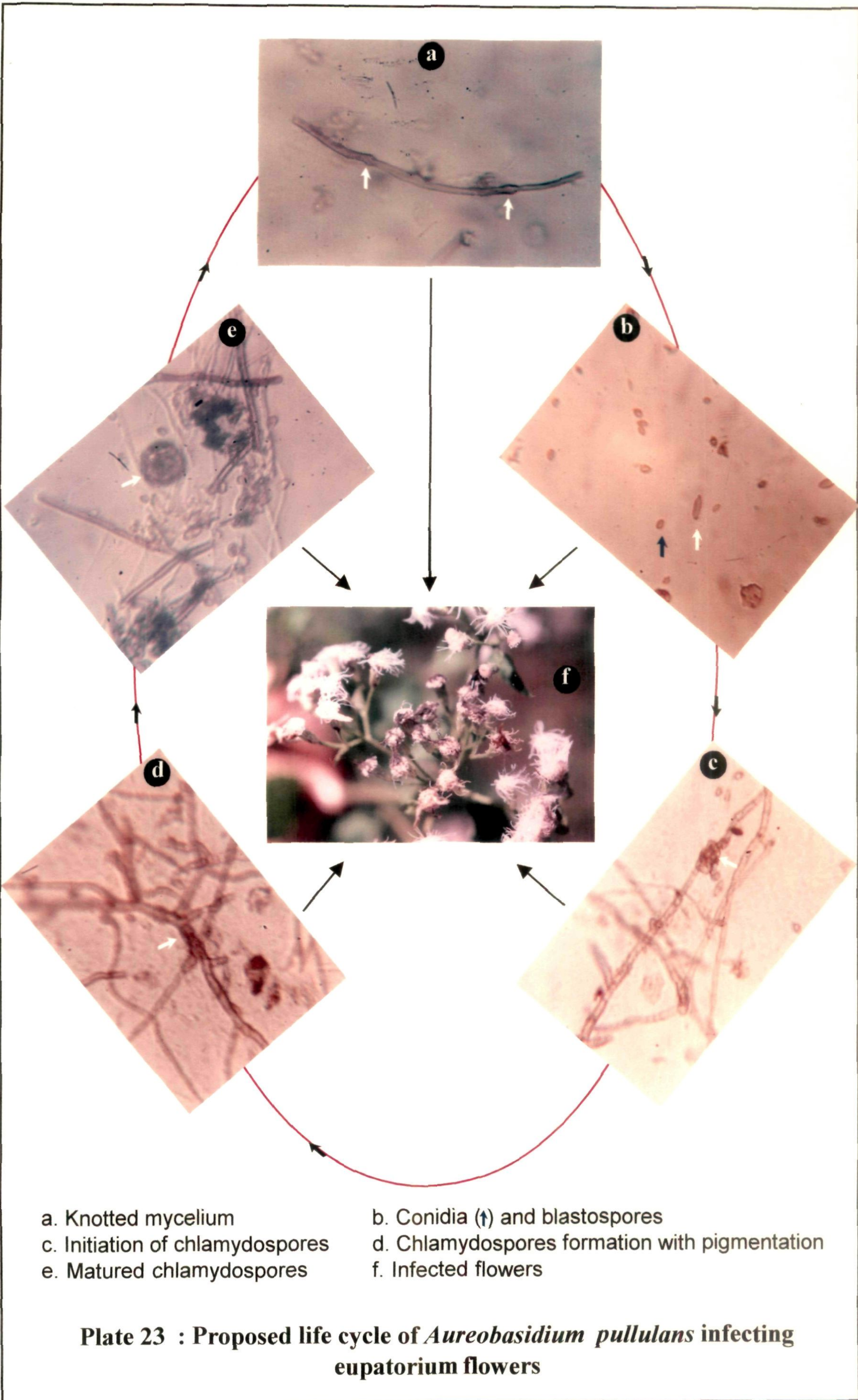


Plate 23 : Proposed life cycle of *Aureobasidium pullulans* infecting eupatorium flowers

degree of infection on the weed. From the results, it can be concluded that, interaction among the pathogens was synergistic which was evident by degree of infection and short incubation period for symptom expression. Degree of infection was maximum when all the pathogens were combined followed by combination of two foliar pathogens than either pathogen used alone (Table 20).

Aureobasidium pullulans caused few mild lesions on foliage which had no profound effect either individually or in combination with foliar pathogens on the development of leaf spots. Hence, it is advisable to combine only two foliar pathogens (*A. alternata* + *C. gloeosporioides*) to induce maximum leaf necrosis in order to manage the weed. With this combination treatment, only eight days of incubation was sufficient to induce maximum disease on the weed under glasshouse conditions and simultaneous applications of two foliar pathogens would be more practical for maximum effectiveness. This may be due to the fact that, *A. alternata* penetrated and infected through the *C. gloeosporioides* infection sites. Thus, *C. gloeosporioides* might have helped the entry of *A. alternata,* comparatively a poor pathogen which infected older plants and leaves. Infection sites of *C. gloeosporioides* prior to *A. alternata* might have helped in passive entry of the latter. Similar results were reported by Crawley *et al* (1985) and Morin *et al.* (1993). Thus, synergistic interaction between these two pathogens made them to attack even younger plants also.

From the stand point of practicality and economics, the infective units of the candidate mycoherbicide must be produced in a timely and cost effective manner. The most suitable infective units are fungal spores. Asexual spores or conidia are generally easiest to produce under experimental conditions, and since spores are the most common mechanisms for natural disease dispersal

and they should serve as the best candidates as infective units of mycoherbicides (Boyette *et al.,* 1979).

Among the several media screened, potato dextrose agar was selected as culture medium, as it was found to be the best medium for all the three potential pathogens to induce sporulation and to obtain inoculum (Boyette *et al.,* 1979).

Two different techniques were employed to mass multiply the pathogens. *A. alternata* was mass multiplied by following submerged culture fermentations (Bowers, 1982) and adopting the method of Conway *et al* (1978) with slight modifications.

Alternaria alternata sporulated heavily in potato dextrose broth compared to other substrates tested for mass production. *C. gloeosporioides* and *A. pullulans* showed excellent sporulation on eupatorium leaf bits within a short incubation period. Though, sporulation efficiency of *C. gloeosporioides* on PDB and eupatorium leaf bits was on par, eupatorium leaf bits were selected as it becomes cost effective and easily available material for mass production. Hence, submerged culture fermentation was followed for *A. alternata* and solid substrate fermentation for *C. gloeosporioides* and *A. pullulans*. This could be a easy and feasible method of mass production.

Alternaria alternata was mass multiplied in PDB for fifteen days followed by homogenization of whole broth which consisted mycelium as well as spores. After harvest of spores and mycelium a sprayable formulation was formulated (Conway *et al.*, 1978).

Colletotrichum gloeosporioides and *A. pullulans* aggressively colonized eupatorium leaf bits (solid substrates) and the quantity (1 kg) of solid substrate

was reduced to one fourth within twenty days of production fermentation. This was due to fast utilization, colonization of leaf material and heavy sporulation. Ultimately, it resulted in powdered form of leaf bits which contained abundant spores as well as mycelial bits. Further, they were formulated as liquid based formulations.

Universal carrier, water was used for spraying the pathogens. Standardized spore load was adjusted by diluting with water. Effect of 3 per cent sucrose as spray additive on disease development was studied and compared with water. Addition of sucrose (3%) to the sprayable formulation gave the best results by hastening the disease development and shortening the incubation period required for epiphytotics. This may be due to the fact that, sucrose helps in uniform and rapid germination of spores as well as support the growth of fungal pathogens (Horsfall and Dimond, 1959).

Efficacy of pathogens under field conditions was studied by spraying mass multiplied pathogens in water with 3 per cent sucrose solution. Standardized spore load of 10^{10} spores/ml, 10^{14} spores/ml and 10^{16} spores/ml for *A. alternata*, *C. gloeosporioides* and *A. pullulans* respectively were maintained during spraying. In the present study, it was noticed that, the foliar pathogens were potential to damage the foliage and the damage was maximum in combi-treatment (*A. alternata* + *C. gloeosporioides*), with per cent disease index 83.36 and short incubation period (20 days) for epiphytotics.

Always spots produced by *C. gloeosporioides* appeared first followed by *A. alternata* infection. Due to this type of synergism they could attack even also the young plants.

Mass inoculum of *A. pullulans* was sprayed in water mixed with 3 per cent sucrose. High humidity was artificially supplied for next 48 h to ensure

disease epiphytotics. It caused cent per cent epiphytotics of floral infection after 20 days of spray inoculation.

Impact of *A. pullulans* on seed biology was studied. It revealed that, even under field conditions *A. pullulans* was able to inhibit seed development. Spraying of *A. pullulans* on flowers resulted in malformed seeds and sterile seeds thus, checking the further spread of the weed and also reducing the weed seed bank.

The present study revealed that, all the treatments were potential to decrease the chlorophyll content significantly at all the stages of growth of the weed. Further, it was observed that, *C. gloeosporioides* reduced total chlorophyll more effectively than *A. alternata* or *A. pullulans*.

It was found that, combination treatments were more superior than individual treatments to damage the total chlorophyll content of leaves. Combination of *A. alternata* + *A. pullulans* + *C. gloeosporioides* reduced the total chlorophyll content to maximum extent (1.03 mg/g fresh. wt) and significantly on par effect was recorded when two foliar pathogens *A. alternata* + *C. gloeosporioides* were combined together (1.04 mg/g fresh wt).

In general, foliar application of pathogens reduced the total chlorophyll content and had direct effect on chlorophyll content as they produced leaf necrosis. These results are in confirmity with the reports of Kempenaar *et. al.* (1996) and Tang *et al.* (1996). The results may be attributed to the fact that, foliar infection leads to abnormalities in the form and structure of chloroplasts. Plants infected by fungi usually exhibit reduced photosynthetic rate, oxidative phosphorylation, hill reaction and carbon dioxide assimilation. These changes may be partially or completely accounted by reduction in chlorophyll content.

The results signified the potentiality of foliar pathogens in combination, in decreasing the total chlorophyll content there by reducing the photosynthetic ability of the weed.

It was observed that, all the pathogens were able to decrease relative water content (RWC %) of the weed. When individual pathogens were sprayed on the weed the reduction in RWC was less compared to decrease in pathogen combinations. Relative water content was reduced to a maximum extent in *A. alternata* + *A. pullulans* + *C. gloeosporioides* combination (50.50%) followed by *A. alternata* + *C. gloeosporioides* combination (51.20%) against control (84.20%) after 21 days. The results are supported by reports of Shabana *et al.* (1996) in case of water hyacinth infected with *A. eichhorniae*. The results may be due to, rupture of epidermis during pathogenesis and enhanced permeability of the tissues, inhibition of stomatal closure or direct water loss through the fungus leads to acceleration of water loss from infected tissues.

It was found that, the accumulation of dry weight was very poor in the infected leaves. Least accumulation of dry matter was recorded in *A. alternata* + *C. gloeosporioides* + *A. pullulans* combination (7.0 g/plant) significantly followed by *A. alternata* + *C. gloeosporioides* combination (7.12 g/plant). This indicated the poor parasitization of *A. pullulans* as a foliar pathogen. However, dry matter accumulation was decreased when *A. pullulans* was inoculated on inflorescence (9.58 g/plant).

As, *A. pullulans* causes black mould of inflorescence, translocation of photosynthates from source to sink may be inhibited. Slight increase in dry matter accumulation was recorded after 14 days, which further decreased after 21 days. This may be attributed to 'feedback inhibition' of synthesized photosynthates.

Overall pathogen infection reduced the dry matter production. The reason for this could be due to decreased assimilatory surface area and photosynthesis.

Spraying of pathogens on eupatorium had marked effect on the assimilatory surface area resulting in significant reduction in the leaf area in all the treatments at all stages. Maximum reduction in leaf area was recorded in *A. alternata* + *A. pullulans* + *C. gloeosporioides* (1122 cm^2/plant) followed by *A. alternata* + *C. gloeosporioides* combination (1131 cm^2/plant). However, the decrease was more in foliar pathogens due to leaf necrosis.

Once the leaf area is reduced, the production and translocation of photosynthates would be decreased, there by decreasing the total dry matter production (Kempenaar *et al.,* 1996).

It was observed that, the specific leaf weight decreased in all the treatments after pathogen spraying. Specific leaf weight was least in *A. alternata* + *A. pullulans* + *C. gloeosporioides* combination (5.96 mg/cm^2) followed by *A. alternata* + *C. gloeosporioides* (6.04 mg/cm^2) against control (9.23 mg/cm^2). Floral infection by *A. pullulans* was also able to reduce specific leaf weight (7.03 mg/cm^2). This may be due to poor photosynthetic rate and reduced dry matter accumulation. In addition to these reasons, leaf necrosis also plays an important role in reduction of specific leaf weight.

In general, the infection by pathogens brings changes in respiratory pathway and photosynthesis which are the vital process taking place inside the plant leading to wide fluctuations in sugars (Farkas and Kiraly, 1962, Kuc, 1966 and Klement and Goodman, 1967). Sugars act as precursor for synthesis of phenols, phytoalexins, lignin and cellulose, which play an important role in plant defense mechanisms.

In the present investigation, sugar content was reduced at all the stages due to pathogen infection. Depletion of total sugar content was dependent on degree of infection. Lowest total sugar was recorded in *A. alternata* + *A. pullulans* + *C. gloeosporioides* combination (11.8 mg/g dry wt) significantly followed by *A. alternata* + *C. gloeosporioides* combination (12.1 mg/g dry. wt) on contrary to highest total sugar content in control (23.5 mg/g dry. wt) at 21 days of spraying. Depletion of total sugar was more in combination treatments than in individual ones (Table 27).

Decline in total sugars in infected plants could be attributed to the competition between pathogen and host for total sugars. Pathogens utilize sugars for their growth and sporulation (Gaumann, 1950). On the other hand the host cells resist these effects and utilise sugars to fuel defense reactions to form poly phenols against infection (Horshfall and Cowling, 1979). Increased respiration may also be a factor for depletion of total sugars in the infected parts (Swamy, 1964).

The enzyme nitrate reductase catalyses the reduction of nitrite to nitrate which is the first step in the assimilation of nitrate by plants.

In the present study, there was gradual decrease in nitrate reductase activity due to infection at all the stages tested. Maximum reduction in nitrate reductase activity was observed in the combination treatment, *A. alternata* + *A. pullulans* + *C. gloeosporioides* (1.53 μ mole No_2 g fr. Wt. min^{-1}) followed by *A. alternata* + *C. gloeosporioides* combination (1.58 μ mole No_2 g fr. Wt. min^{-1}). Extent of decrease of NRA was more in combination treatments than in individual treatment. However, all the treatments significantly reduced the NRA content.

The impairment in the activity of nitrate reductase in *C. odorata* possibly might affect the nitrogen metabolism. If NRA is inhibited, the nitrate fraction of nitrogen accumulates in the plant system and becomes phytotoxic. Thus, both foliar and floral pathogens of *C. odorata* could impair nitrogen metabolism through NRA.

Among all the biochemical components of different hosts, phenolics standout as most important components in imparting resistance to several plant diseases. In the present study, it was observed that, total free phenolics increased in plants due to infection. Total free phenolics increased to a maximum extent in *A. alternata* + *A. pullulans* + *C. gloeosporioides* (14.21 mg/g dry. Wt) followed by *A. alternata* + *C. gloeosporioides* (14.03 mg/g dry wt), where as total free phenolics increased to a lesser extent when pathogens were applied individually. In general, the infection resulted in increase in total free phenols in the plants. Similar results were reported by Friend (1981) and Shabana *et al.* (1996).

The increase in total phenolics content could be due to a number of factors including enhancement of synthesis or translocation of phenolics to site of infection and hydrolysis of phenolic glycosides by fungal glycosidases to yield free phenolics.

Parasitic organisms that direct the synthesis of their own constitutive proteins would be expected to interfere with host protein metabolism and aminoacid synthesis. Amino acids may act as inhibitory to the activity of pathogen or may act as precursors of various fungitoxic compounds, particularly phenolics.

In the present investigation, free aminoacid content in the infected plants decreased significantly at all stages. Pathogens in combination reduced the free

aminoacids more effectively than individual pathogens. Reduction was maximum in *A. alternata* + *A. pullulans* + *C. gloeosporioides* combination (1.51 mg/g dry. wt) followed by *A. alternata* + *C. gloeosporioides* combination (1.82 mg/g dry. wt). Free aminoacid content was maximum in control (4.11 mg/g dry. wt) at 21 days of spraying. However, the decrease was at varying levels depending upon the pathogen association. Decline in free amino acid content in infected plants may be due to inability of infected leaves to synthesize carbohydrate which are essential for synthesis of amino acids or it may be due to utilization of aminoacids for nutritional purpose by the pathogens. These results are in accordance with the findings of Shabana *et al.* (1996).

The data indicated that, photosynthetic rate was decreased in plants infected with pathogens, at all the stages. Photosynthetic rate of eupatorium was checked to a maximum extent in *A. alternata* + *A. pullulans* + *C. gloeosporioides* combination (3.00 μ mole CO_2 dm^2/S_1) followed by *A. alternata* + *C. gloeosporioides* combination (3.15 μ mole CO_2 dm^2/S_1). Floral infection due to *A. pullulans* resulted in decreased photosynthetic rate (5.15 μ mole CO_2 dm^2/S) after 21 days however, on 14th day of spray inoculation, there was slight increase in photosynthetic rate.

Transpiration rate was decreased in all the treatments at all stages. It was reduced to a maximum extent in *A. alternata* + *A. pullulans* + *C. gloeosporioides* (3.15 μ mole H_2Om^2/S^1) followed by *A. alternata* + *C. gloeosporioides* (3.45 μ mole H_2Om^2/S^1) after 21 days. Transpiration rate was greatly ceased, when *A. pullulans* was on flowers (4.51μ mole H_2Om^{-2}/S^{-1}). Yang and Guo (1991) reported decreased transpiration and photosynthetic rates in *Eupatorium adenophorum* infected with Mycovellosiella eupatori odorati

reported similar results. The experimental results were in agreement with reports of Tang *et al.* (1996) and Kempenaar *et al.* (1996).

These results may be attributed to the fact that, photosynthetic capacity of the plant is mainly controlled by other biophysical parameters like transpiration rate and temperature as it involves the exchange of gases. Fungal pathogens seem to affect the photosynthetic process by affecting the chloroplasts, chlorophyll or the enzyme system associated with the process of photosynthesis and respiration.

Decline in chlorophyll content and transpiration rate is correlated with the decline in the photosynthesis. If the toxins are produced by the fungus, they can also affect the chloroplasts and rate of photosynthesis.

Increased respiration is a common feature of diseased plants. The increase in the rate of respiration in the infected tissues are a joint contribution of the host and pathogen (Dasgupta, 1988). In the present findings the leaf temperature was slightly more in diseased plants when compared to the leaf temperature in control plants. Leaf temperature was slightly increased when individual pathogens were sprayed without any significant difference among treatments. Leaf temperature was maximum in *A. alternata* + *C. gloeosporioides* + *A. pullulans* combination (32.8 °C) followed by *A. alternata* + *C. gloeosporioides* combination (32.4°C). This may be due to high respiration of both the host as well as the pathogens. Dasgupta (1988) highlighted the increase in respiration due to diseases in turn increase in leaf temperature by 0.3 to 0.7 per cent.

The efforts of the present investigation contributed in identification and tagging of the potential pathogens *viz.*, *A. alternata, C. gloeosporioides* and *A. pullulans* as foliar and floral pathogen of *C. odorata*, respectively. Foliar spray

inoculation of *A. alternata* @ 10^{10} spores per ml and *C. gloeosporioides* @ 10^{14} spores per ml on 2½ months old seedlings damaged the leaves by reduced photosynthesis inturn reduced productivity of the weed. However, *A. pullulans* @ 10^{16} spores per ml was found more promising as it caused seed abortion and malformed seeds. Thus, further spread of the weed and invasion of virgin lands by the weed would be checked effectively.

Future line of work

The present investigation has given an insight into management of *Chromolaena odorata* (Eupatorium) by using mycoherbicides. However, the following objectives are suggested for future research work.

1) Mycoherbicidal efficacy under field condition largely depends on weather parameters. Hence a detailed study of correlation between weather parameters and disease epiphytotic in weed host population needs further attention,

2) The study on shelf life and stability of potential mycoherbicides is required so as to maintain viability of spores over a wide range of handling and storage conditions,

3) To improve viability and efficacy of mycoherbicides, improved mass production technologies need to be developed,

4) To avoid artificial supply of dew period, formulations may be developed that prevent drying / promote moisture retention of fungal spores,

5) Large scale field application, field demonstration may be carried out and the products may be patented for large scale usage by the farmers in endemic areas, and

6) *Aureobasidium pullulans* proved to be a floral pathogen checks the further spread of the weed and hence it needs detailed study.

SUMMARY

VI. SUMMARY

Chromolaena odorata (L.) King and Robinson is an alien, obnoxious perennial shrubby weed abundantly found in the deforested areas, marginal lands, range lands and by the roadsides. *C. odorata* (Eupatorium) is a weed of the forests, pastures and plantation crops and it is currently threatening the ecological integrity of India. The present investigation was carried out with special reference to its biological control by using fungal pathogens and their mass production.

The experiments were carried at Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad and the field experiments at the Farm Forestry Station, University of Agricultural Sciences, Dharwad during 1995-97.

A survey was carried out in eupatorium endemic parts of Karnataka state viz., Belgaum, Dharwad, Uttar Kannada and Shimoga districts during 1994-95, which resulted in isolation of eighteen fungal isolates. *Alternaria alternata* (Fr.) Keissler caused leaf spots and necrosis of affected parts which was isolated from Belgaum, Khanapur, Kalaghatagi, Sirsi, Yellapur, Hubli, Shimoga and Hosanagar. *Colletotrichum gloeosporioides* Penz. caused leaf spots which was isolated from Dharwad and Prabhunagar.

Bipolaris sp. was recorded from Sirsi and Londa which caused leaf spots. The other foliar pathogens isolated were *Cladosporium spherospermum* Penz., *Beltrania rhombica* Penz., *Phoma eupatorii* died, *Phomopsis* sp. and *Phyllosticta* sp. from Dharwad, Sagar, Mundagod, Sirsi and Haliyal talukas, respectively. Thus nine different genera *viz., Alternaria, Aureobasidium, Beltrania, Bipolaris* sp., *Cladosporium, Colletotrichum , Phoma, Phomopsis* sp. and *Phyllosticta* sp.

were isolated from diseased leaves, except *Aureobasidium pullulans* (deBary) Arnaud which was isolated from infected flowers. *A. pullulans* was the only one floral pathogen collected during survey work which caused black mould of inflorescence. All the isolated organisms were found pathogenic at varying degrees and satisfied the Koch's postulates.

Among the eighteen isolates screened under glass house conditions seven isolates viz., *Alternaria alternata* (Yellapur isolate), *Aureobasidium pullulans, Colletotrichum gloeosporioides* (Dharwad and Prabhunagar isolates), *Phoma eupatorii* (Mundagod), *Phomopsis* sp. (Sirsi) and *Phyllosticta* sp. (Haliyal) were selected based on their per cent disease index and virulence. The remaining isolates were deleted from the study.

Further, based on *in vitro* sporulation and growth studies of seven isolates on artificial media, *C. gloeosporioides* and *A. pullulans* were selected as they showed profused growth and excellent sporulation and *A. alternata* exhibited good sporulation with low mycelial growth. Remaining isolates failed to sporulate, though they showed profuse mycelial growth. Hence, *A. alternata, A. pullulans* and *C. gloeosporioides* were selected as potential virulent pathogens of eupatorium weed and remaining isolates were rejected in further studies.

Among the different solid media, potato dextrose agar was most suitable for all the three potential virulent pathogens. Next best was Sabouraud's dextorse agar followed by Richards's agar medium. Potato dextrose agar supported maximum sporulation, followed by host extract agar + 1% sucrose medium.

Maximum mycelial growth of *C. gloeosporioides* and *A. pullulans* was recorded on 10th day of seeding in potato dextrose broth whereas, *A. alternata*

required 12 days of incubation for maximum growth. Hence, these periods were considered while harvesting the pathogens in nutritional and physiological studies.

Among the liquid media, potato dextrose broth was the most favoured medium for potential pathogens. Next best media for *A. alternata* was Richards's medium followed by Sabouraud's broth. Second best medium for *A. pullulans* and *C. gloeosporioides* was Sabouraud's broth. Among synthetic media, Richard's medium was best for both the pathogens.

Temperature of 25°C was optimum for maximum growth of *A. alternata*, followed by 30°C. Optimum temperature of 30°C was found to be suitable for maximum growth of *C. gloeosporioides* and *A. pullulans* however, the pathogens showed good growth even at 25°C.

Alternaria alternata required pH 5.50 for maximum growth, whereas, *C. gloeosporioides* showed maximum growth at pH 6.50. *A. pullulans* showed high plasticity adapting to a wide pH range 4.0 to 8.0. Maximum growth was recorded at pH 6.0 and 8.0.

Among the different carbon sources, dextrose was the most favoured for the growth of all three potential pathogens. Next best carbon source was glucose for growth of *A. alternata* and *A. pullulans.* Sucrose was the second best carbon source for growth of *C. gloeosporioides*.

Among the different nitrogen sources, peptone supported the maximum growth of all the pathogens. Second best nitrogen source for the growth of *A. alternata* and *A. pullulans* was potassium nitrate and tyrosine for *C. gloeosporioides*.

Toxin studies indicated that, *A. alternata* and *A. pullulans* were able to produce toxins against eupatorium weed and tomato cuttings. Whereas *C. gloeosporioides* failed to produce toxins against the weed and tomato cuttings. Production of toxins by *A. alternata* and *A. pullulans* was visualised by the necrosis, drying and wilting of the tomato and eupatorium cuttings.

Host range study of potential pathogens of eupatorium indicated that, the pathogens did not infect the economic hosts. However, the pathogens failed to infect *Parthenium hysterophorus* and *Lantana camera*. All the plantation crops tested viz., arecanut, coconut, betelvine, cardamom, cocoa, coffee, banana and cashew were healthy and showed no symptoms due to infection. Similarly the field crops (paddy, cotton, sunflower and cowpea) and forest crops (teak, eucalyptus and bamboo) were free from infection.

A second year survey and surveillance carried out during 1996-97 in Dharwad, Prabhunagar, Mundagod and Yellapur confirmed the endemic nature of *C. gloeosporioides* (Dharwad and Prabhunagar), *Aureobasidium pullulans* (Mundagod) and *Alternaria alternata* (Yellapur) respectively. Thus all three potential pathogens selected in the present investigation proved their endemic nature against eupatorium weed.

Among the different inoculation techniques tested, only spraying technique was useful to cause the disease on eupatorium. Pathogens failed to induce disease symptoms when inoculated following seed inoculation or soil inoculation or stem inoculation method. Hence, in all further experiments 'spraying technique' was followed. To create artificial disease epiphytotics and to induce maximum disease on the weed all the pathogens were sprayed at their optimum spore dose and at vulnerable stage.

Inoculum density for each pathogens was standardized, optimum spore concentration for *A. alternata, C. gloeosporioides* and *A. pullulans* was 10^{10} spores/ml, 10^{14} spores/ml and 10^{16} spores/ml respectively, to cause maximum disease on the weed.

It was established that, young eupatorium plants (two months old) were susceptible to *C. gloeosporioides.* As the plants aged, susceptibility to *C. gloeosporioides* was decreased. *A. alternata* induced maximum disease on three months old weed plants and it favoured old leaves and aged plants for infection.

Spraying of *A. pullulans* on flowers resulted in black mould. Disease was maximum from flower opening to full bloom. Infection resulted in malformed sterile seeds and premature dropping of flower buds. Pathogen could not infect buds and infection was superficial on matured seeds. Hence, it was concluded, to inoculate *A. pullulans* @ 10^{16} spores/ml at flower opening stage or full bloom stage to check seed development and further spread of the weed. Symptoms were noticed eight days after inoculation.

Alternaria alternata caused leaf spots with typical target board symptoms. The spots were surrounded by yellow halo. Later the spots coalesced, which resulted in drying of leaves and defoliation. The spots were more distributed on older leaves. Some times veinal necrosis was also noticed which might be due to toxin production by *A. alternata*. All these symptoms were noticed on 12th day after spraying the pathogen under glasshouse conditions.

Colletotrichum gloeosporioides caused leaf spots and it infected younger leaves. The leaf spots were black, irregular and minute to large distributed in the centre or margin of leaf. Later the spots coalesced and covered large

patches. Under severe conditions, 'shot hole' symptoms were noticed. These symptoms were noticed ten days after inoculation of the pathogen under glasshouse conditions.

Though, *A. pullulans* was a floral pathogen, it caused a few mild lesions on leaves. Under glass house conditions, combined inoculation of pathogens on the weed indicated that, interaction between the pathogens was synergistic. This was evident by increased per cent disease incidence in combined treatments over individual treatments. When all the pathogens were integrated together and inoculated on foliage (*A. alternata* + *A. pullulans* + *C. gloeosporioides*) disease was the maximum (94.00%) followed by inoculation of two foliar pathogens together (*A. alternata* + *C. gloeosporioides*) with 92.00 PDI. *A. pullulans* was a aggressive invader of flowers which resulted in cent per cent infection of inflorescence leading to aborted seeds.

For mass production of pathogens 'agar slants' were standardised as 'stock culture' and potato dextrose agar as 'culture medium'.

Among different substrates tested for mass production of pathogens, PDB supported the maximum growth and sporulation of *A. alternata* as compared to other substrates. So, *A. alternata* was multiplied in P.D.B. and the methodology developed was 'submerged fermentation technique'. *A. pullulans* and *C. gloeosporioides* sporulated heavily on eupatorium leaf bits as compared to other substrates and they were mass multiplied by adopting 'solid substrate fermentation technique'.

To improve spray efficacy, addition of three per cent sucrose was found to be suitable, as it advanced the disease epiphytotics by three to five days.

Spraying of pathogens in combination under field condition resulted in the maximum disease than in individual treatments. Results showed the maximum disease (85.98 PDI) epiphytotics at twenty days after spraying when all the pathogens (*A. alternata* + *A. pullulans* + *C. gloeosporioides*) were integrated followed by integration of two foliar pathogens (*A. alternata* + *C. gloeosporioides*) (83.36 PDI). As influence of *A. pullulans* on disease epiphytotics was negligible, it is advisable to delete *A. pullulans* from the combination (*A. alternata* + *C. gloeosporioides* + *A. pullulans*) for foliar spraying.

Aureobasidium pullulans was a promising floral pathogen and caused cent per cent black mould of flowers and disease epiphytotics was created 18 days after spraying. Infection on just opening flowers resulted in either premature dropping or complete destruction of flowering parts. Infection at full blooms resulted in sterile and malformed or aborted seeds.

Inoculation of all the pathogens individually or in combination changed the physiological and biochemical processes of eupatorium. The maximum reduction in total chlorophyll, relative water content, dry matter accumulation and size of assimilatory surface area was recorded when all the pathogens were combined (*A. pullulans* + *A. alternata* + *C. gloeosporioides*) followed by foliar application of two pathogens in combination i.e. *A. alternata* + *C. gloeosporioides*.

Similarly, reduction was observed with respect to other physiological parameters *viz.*, specific leaf weight, photosynthetic rate, transpiration rate, total sugar content and free amino acids. However, infection in plants resulted in accumulation of total free phenols and increase in leaf temperature. This effect was more when all the pathogens were combined and inoculated (*A. alternata* + *C. gloeosporioides* + *A. pullulans*) followed by foliar pathogens combination (*A. alternata* + *C. gloeosporioides*).

Acc. No. 6740

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*Original not seen

APPENDIX

Appendix 1: Meteorological data for the cropping season 1996-97 and average of 45 years (1950-95) as recorded at the Meteorological observatory, University of Agricultural Sciences, Dharwad

Months	Average rainfall (mm)		Mean temperature (°C)				Mean relative humidity (%)	
			Maximum		Minimum			
	1950-95	1996-97	1950-95	1996-97	1950-95	1996-97	1950-95	1996-97
July	164.58	184.40	25.90	26.20	20.29	20.90	89.04	87.00
August	110.72	50.50	26.22	28.10	20.27	20.70	87.04	85.00
September	97.95	121.60	28.56	28.20	19.76	20.50	81.65	82.00
October	129.12	127.60	29.63	29.30	19.08	19.90	77.37	81.00
November	19.13	81.00	28.85	29.10	15.97	15.80	73.64	81.00
December	1.66	0.00	28.55	28.70	13.67	13.50	71.42	77.00
January	0.56	0.00	29.36	30.10	14.76	14.60	60.52	75.00
February	0.19	0.00	32.24	32.50	15.16	16.30	52.61	71.00
March	6.80	5.00	34.82	34.70	18.30	19.60	54.96	64.00
April	49.52	13.20	36.29	36.70	20.52	20.30	58.61	60.00
May	85.16	65.60	34.56	37.40	21.25	21.10	65.09	63.00
June	107.95	130.10	29.23	30.20	20.80	21.40	79.97	78.00

MANAGEMENT OF EUPATORIUM [*Chromolaena odorata* (L.) King and Robinson] WEED THROUGH MYCOHERBICIDES

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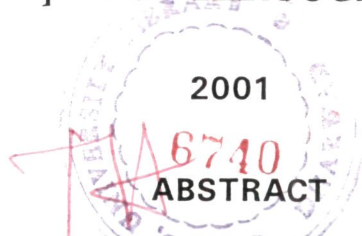

ABSTRACT

A survey was conducted in eupatorium endemic districts of Karnataka *viz*., Belgaum, Dharwad, Uttar Kannada and Shimoga during 1994-96 to exploit the mycoherbicidal potential of the pathogens associated with the weed.

Nine different pathogens encompassing eighteen fungal isolates were isolated from the weed viz., *Alternaria alternata, Aureobasidium pullulans, Beltrania rhombia, Bipolaris* sp., *Cladosporium sphaerospermum, Colletotrichum gloeosporioides, Phyllosticta* sp., *Phoma eupatorii* and *Phomopsis* sp. Among these *A. pullulans* was the only floral pathogen causing black mould and remaining ones were foliar pathogens, causing leaf spots.

Alternaria alternata (Yellapur), *C. gloeosporioides* (Prabhunagar) and *A. pullulans* (Mundagod) were highly virulent and used as potential mycoherbicides.

The best solid medium for growth and sporulation was Potato dextrose agar. Optimum temperature and pH for the maximum growth of *A. alternata* was 25°C and 5.50, respectively. For *C. gloeosporicides* 30°C and pH 6.50 were optimum. *A. pullulans* favoured 30°C and pH 6.0 and 8.0.

Alternaria alternata and *A. pullulans* produced toxins against the weed whereas *C. gloeosporioides* did not. Host range study indicated that all the plantation, field crops and forest trees tested were immune to pathogens.

Foliar spraying of *C. gloeosporioides* @ 10^{14} spores/ml on two months old plants and *A. alternata* @ 10^{10} spores/ml on 3 months old plants induced the maximum leaf spot disease.

Spraying of *A. pullulans* @ 10^{16} spores/ml at flower opening / full bloom resulted in maximum black mould infection.

Interaction between the pathogens was synergistic. A field experiment was conducted at Farm Forestry Station, UAS, Dharwad during 1997 and combi spraying of pathogens, *A. alternata* + *C. gloeosporioides* + *A. pullulans* on eupatorium plants resulted in maximum PDI, followed by combi spraying of *A. alternata* + *C. gloeosporioides*.

Aureobasidium pullulans caused cent per cent flower infection rendering sterile and malformed seeds. Spraying of pathogen changed the physiological, biophysical and biochemical processes of eupatorium.