Aureobasidium pullulans, a potential mycoherbicide for biocontrol of eupatorium [*Chromolaena odorata* (L.) King and Robinson] weed

Chromolaena odorata commonly known as eupatorium, is an alien, obnoxious and aggressive weed. It has occupied pastures, marginal lands, open areas, dry deciduous forests and interior shrub jungles, where it is highly competitive and does not let other flora grow. It is a menace in plantations, agriculture and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic potentialities and growth inhibitors[1]. The weed poses a grave threat to the fragile biodiversity of the Western Ghats, where it is competitively replacing the existing indigenous rich flora, thereby creating ecological imbalance[2]. The Forest Department of Karnataka spends several lakhs of rupees annually to clear this weed in nurseries and young plantations, but the problem has remained unsolved and also severe. The rapid spread of the weed is due to extensive seed production, which is estimated to be 93,000–160,000 seeds/plant[3] and also wind dispersal of seeds[4]. All these points highlight that eupatorium is a threat to agriculture and environment. Hence there is an urgent need to manage weed growth and its spread so as to maintain ecological integrity.

In resolving the problems posed by eupatorium, current control methods are not capable of providing long-lasting solutions since manual control is uneconomical due to resprouting and perennial nature of the weed. Herbicidal control is not only a costly affair but also causes environmental pollution, human and animal health hazards. In this background the only way left is to adopt an economically sound and environmentally safe practical method, i.e. biological control. However, biocontrol attempts to manage the weed through the insect, *Pareuchaetes pseudoinsulata* became futile due to its poor establishment under field conditions in Karnataka[5]. Hence the present study was aimed to exploit the fungal pathogens associated with eupatorium as mycoherbicides and to manage the weed effectively.

A field survey was conducted in agriculture and forest areas of eupatorium endemic districts in Karnataka, viz. Shimoga, Belgaum, Uttar Kannada and Dharwad to collect diseased specimens. The organisms were isolated from the diseased parts of the weed by following standard tissue isolation procedure on potato dextrose agar (PDA) medium. Isolated cultures were sent to Agharkar Research Institute, Pune for identification. The survey resulted in isolation of eighteen different isolates distributed in nine different genera (Table 1). All organisms were foliar pathogens except *A. pullulans*, which was a floral pathogen. All the isolates satisfied Koch's postulates and hence proved to be pathogenic to the weed. During survey, eupatorium flowers showing sooty mould symptoms were collected from Mundagod taluk, Uttar Kannada district. The organism associated with diseased flowers was identified as *Aureobasidium pullulans* (de Bary) Arnaud., a facultative parasite. It is a member of Fungi Imperfecti, included in the order Moniliales, family Dematiacae[6]. To confirm the pathogenic nature of *A. pullulans*, it was multiplied on PDA and spore suspension was prepared with sterile distilled water. The suspension was sprayed on flowers both under glasshouse and field conditions. It caused sooty mould and premature dropping of flower buds, and thus proved to

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

Location	Pathogen isolated	Plant part/part affected
Belgaum		
Belgaum	*Alternaria alternata* (Fr.) Keissler	Leaf lamina
Khanapur	*A. alternata* (Fr.) Keissler	Leaf lamina
Londa	*Bipolaris* sp.	Leaf lamina
Dharwad		
Dharwad	*Colletotrichum gloeosporioides* (Penz.) Sacc.	Leaf lamina
Prabhunagar	*Cladosporium sphaerospermum* Penz.	Leaf lamina
	C. gloeosporioides	Leaf lamina
Hubli	*A. alternata* (Fr.) Keissler	Leaf lamina
Kalagatagi	*A. alternata* (Fr.) Keissler	Leaf lamina
Uttar Kannada		
Haliyal	*Phyllosticta* sp.	Leaf lamina
Mundagod	*Phoma eupatorii* Died	Leaf lamina
	Aureobasidium pullulans (deBary) Arnaud	Inflorescence
Sirsi	*A. alternata* (Fr.) Keissler	Leaf lamina
	Phomopsis sp.	Leaf lamina
	Biopolaris sp.	Leaf lamina
Yellapur	*A. alternata* (Fr.) Keissler	Leaf lamina
Shimoga		
Hosanagara	*A. alternata* (Fr.) Keissler	Leaf lamina
Sagar	*Beltrania rhombica* Penz.	Leaf lamina
Shimoga	*A. alternata* (Fr.) Keissler	Leaf lamina

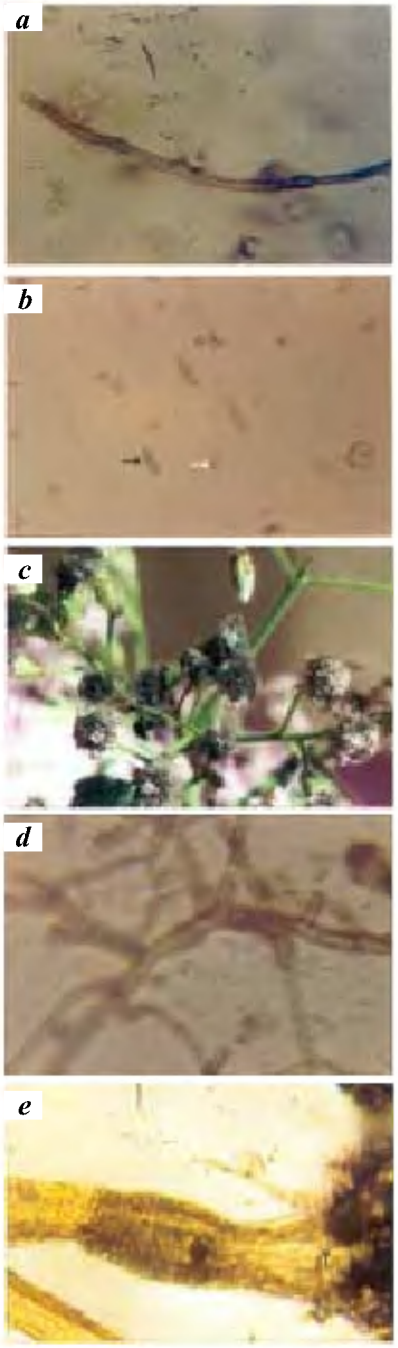

Figure 1. *Aureobasidium pullulans* a promising mycoherbicide of eupatorium weed. ***a***, Mycelium; ***b***, Blastospores and conidia; ***c***, Infected flowers; ***d***, Chlamydospores and ***e***, Colonized flower.

be a pathogen of eupatorium flowers. Further, the organism was reisolated and pure culture was obtained. It exhibited diverse morphology by producing mycelium, blastospores, conidia and chlamydospores (Figure 1 *a*–*c*). This is a new report of *A. pullulans* as a floral pathogen of eupatorium. Further, the experiment was continued to exploit this pathogen as a mycoherbicide.

Table 2. Colony diameter and sporulation capacity of *A. pullulans* on different media

Media	Colon diameter (mm)	Relative sporulation
Selective		
Czpapek's agar	30	4
Czpapek's Dox's agar	50	4
Richards's agar	70	4
Sabouraud's dextrose agar	78	4
Tochinais agar	70	4
Non-selective		
Host extract agar + 1% sucrose	55	4
Host extract agar	40	4
Oat meal agar	75	3
Potato dextrose agar	85	4
Mean	61.67	

Relative amount of sporulation

No. of spores/microscopic field	Grade
>70	4
50–70	3
20–50	2
>20	1
0	0

Several selective and non-selective media were screened to find out the best medium for growth and sporulation of *A. pullulans*. The composition and procedures for preparation of the media were followed, as explained by Tuite[7]. Eupatorium leaf extract agar medium was prepared by boiling 200 g leaf bits in 500 ml of water for 10 min and filtering through muslin cloth. Twenty ml of agar was melted separately in 500 ml of water. Both the solutions were mixed and the volume was made up to 1000 ml.

Fifteen ml of each medium was poured into each of 90 mm sterilized petri plates. The five mm mycelium disc of *A. pullulans* was aseptically inoculated into each petri plate and incubated at 27 ± 1°C. Observation on colony radial growth and sporulation capacity of *A. pullulans* was taken when the maximum growth was attained in any one of the media tested. *A. pullulans* exhibited maximum radial growth on PDA (85.00 mm) and sporulated heavily on all the selective and non-selective media, except oat meat agar medium (Table 2). Hence, PDA was used for culturing *A. pullulans*.

A second year survey and surveillance (1998–99) carried in Mundagod taluk confirmed the endemic nature of *A. pullulans* on eupatorium. An endemic pathogen may be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particular susceptible stage of weed growth[8]. Different levels of spore concentration were assayed to induce maximum disease on the weed. It was found that a spore load of 10^{16} spores/ml was required to cause maximum disease. LD_{50} value was also standardized and it was found to be 10^{10} spores/ml.

A critical consideration in the development of a mycoherbicide for weed control is the determination of host range. Irrespective of potential benefits of pathogens, the safety of non-target cultivated plants and wild plants must be ensured prior to its field release as a mycoherbicide. To satisfy this criterion, host safety of *A. pullulans* was tested following modified centrifugal phylogenetic strategy[9]. The plant species were tested *in vitro* through detached leaf technique. All the crops and tree species tested, viz. cotton, paddy, sunflower, cowpea, arecanut, coconut, pepper, beetlevine, banana, cardamom, cocoa, cashew, coffee, eucalyptus, teak and bamboo were immune to the pathogen. Thus, *A. pullulans* proved to be a safe mycoherbicide without affecting field crops,

Table 3. 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 inability of the pathogen to enter inside the epidermis.

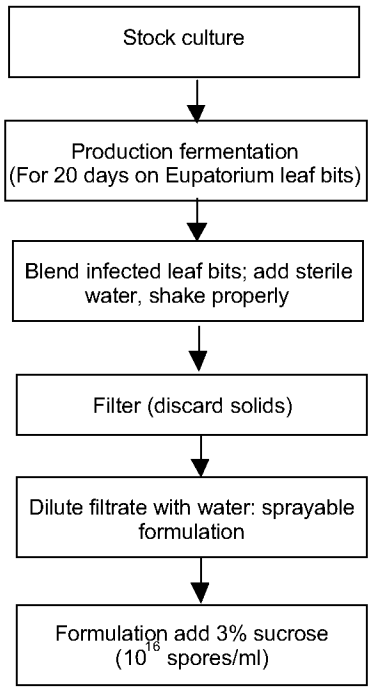

Figure 2. Mass production of *Aureobasidium pullulans* (solid substrate fermentation).

Table 4. Effect of *A. pullulans* on seed viability of *C. odorata*

No. of cypsella/ flower	Per cent germinated seeds	Per cent mal formed seeds	Per cent sterile seeds
30	0	0	100
35	5.7	8.57	85
32	0	93.75	6.25
34	0	11.76	88.23
28	0	35.71	64.28

plantation crops and forest trees that share a common ecosystem with the weed. However, the pathogen was unable to infect the other major weeds, viz. *Lantana camara* and *Parthenium hysterophorus* (Table 3).

A. pullulans was effectively mass multiplied on eupatorium leaf bits following solid substrate fermentation technique[10] (Figure 2) The pathogen grew profusely and sporulated heavily on eupatorium leaf bits. Further, the substrate was mixed with sterile water and blended. The spore suspension was prepared and adjusted to a spore load of 10^{16} spores/ml to get the maximum infection. To advance the disease development and to improve mycoherbicidal efficacy, 3% sucrose was added as a spray additive to the spore suspension.

A field experiment was conducted on naturally grown eupatorium weed at Farm Forestry Research Station, University of Agricultural Sciences, Dharwad during 1998–2000. Spore suspension of *A. pullulans*@10^{16} spores/ml with 3% sucrose was sprayed on eupatorium flowers at flower opening and full-bloom stage in four different experimental sites of 4×3 m^2 area. Spraying was carried out during evening hours and 48 h dew period was maintained for plants sprayed with *A. pullulans*. Controlled plants were sprayed with water + 3% sucrose. The pathogen

colonized the reproductive parts and caused sooty mould of flowers after 18 days of spraying (Figure 1*d* and *e*). Due to infection premature dropping of flower buds was also noticed. In the natural environment, pathogen colonized the reproductive parts and caused sooty mould of flowers after 18 days of spraying. During infection process *A. pullulans* produced blastospores, conidia and chlamydospores. The secondary spread of disease was through wind-borne conidia/blastospores.

Further, impact of *A. pullulans* infection on seed viability was studied by seed germination test. Infection resulted in production of sterile and malformed seeds (Table 4; Figure 1*f–h*). Thus *A. pullulans* as a floral pathogen inhibited floral development, embryo development, seed filling and rendered the seeds sterile. It was noticed that all its morphological stages were infective. As the pathogen produces chlamydospores, it may be an added advantage to carry over the pathogen to the next season and to formulate it at commercial scale. Spraying of *A. pullulans* has no deleterious effects on other plant species which share the common ecological niche, as was confirmed by host safety test. However, a detailed study on its ecology needs further attention. In view of copious seed production, wind dispersal nature and invasive capacity of eupatorium weed even in hilly areas and plains, exploitation of *A. pullulans* as a floral pathogen appears to be a hopeful potential mycoherbicide of eupatorium, as it causes sterile and malformed seeds, and thereby checks the weed seed production and its spread to virgin lands. Research is in progress to develop formulation of *A. pullulans* as a mycoherbicide for large-scale field application.

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Coal-fire detection and monitoring in Raniganj coalfield, India – A remote sensing approach

Raniganj coalfield, West Bengal is the largest coalfield in India, belonging to the Gondwana Super Group[1]. Asansol, situated about 210 km NW of Kolkata (Figure 1), is the main town in this coalfield. Mining in this region dates back to the British period. Initially coal mining was confined to open cast mines only, but gradually it was extended to the underground also. Coal-fire in Raniganj coalfield is either because of fire infection from adjacent fire-affected coal seams or anthropogenic activities or spontaneous combustion of coal. Oxidation of coal is an exothermic process and if the heat generated is allowed to accumulate, then the accumulated temperature ignites the coal. This natural process is called spontaneous combustion and is one of the major causes of coal fire in Raniganj coalfield. Thus India is losing good quality coal prior to its exploitation. Hence, there is need for detection and monitoring of coal fires in coalfields in order to control them effectively.

Remote sensing technique in thermal band offers a cost-effective and time-saving technology for mapping various geoenvironmental features like coal fires, forest fires, oil-well fires, volcanic eruptions, etc.[2]. These features are identified in band-6 (10.4–12.5 μm) as high-temperature anomalous areas[3] because hot bodies on the surface of the earth mostly emit radiation in this band Landsat-5 Thematic Mapper (TM) daytime digital data were used for this study. Night-time data were not considered for this study because ground control points, which are required for registration of the satellite image to the base map, are difficult to identify. The limitation of daytime data is that thermal anomalies represent partial underground coal fires with partial solar heating of non-burning coal seams and black shale with higher emissivity[4]. Winter-season data were selected for this study as the anomaly between fire and non-fire zones will be conspicuous. Topographic factors like aspect, slope angle and morphology of the area have a strong relation with reflectance and radiant temperature of an object[4]. Raniganj coalfield has a subdued topo-

Figure 1. Location map of Raniganj coalfield, West Bengal.