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Exploitation of phytopathogenic fungal diversity for the development of bioherbicides

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President 2013-14

At the very outset I would like to thank the members of the Executive Committee and all the members of Mycological Society of India for the honour they have bestowed upon me by electing me the President of the Society for 2013-14. I consider this a great privilege and elated to be the President of an old, prestigious society whose foundation was laid in the year 1973. I am lucky to have been associated with the Society since 1976 when I was a research student. I owe a gratitude to my teacher Prof. R.S. Mehrotra, a leading mycologist and plant pathologist, for developing interest to work on biodiversity of fungi.

I have chosen the topic of my today's talk not because of my long association with the subject of biocontrol of weeds with fungal pathogens and fungal diversity but to stimulate research in the applied area so that budding scientists working in diverse areas, such as weed science, plant pathology, fungal diversity, biotechnology, microbiology and environmental science, could bring out novel products for controlling weeds by using biocontrol agents and reducing the dependence on chemical herbicides.

INTRODUCTION

Weeds, one of the major kinds of pests, can reduce crop yield by as much as 12 % which results to $ 32 billion as a whole. Management of weeds by herbicides annually account for over $ 14 billions (Kiely *et al*., 2004). Chemical weed control is not an ideal option in organic cropping systems. The application of biological control for management of weed populations using biocontrol agents (BCAs), particularly fungal pathogens, has gained acceptance as a safe and environmentally sound additional approach which minimizes hazards resulting from herbicide residue to human and animal health, and to the biodiversity (Mortensen, 1988; Rosskopf *et al*., 1999; Aneja, 1999; 2009; Boyetchko *et al*., 2002; Boyetchko and Peng, 2004). The intentional use of phytopathogens that are mass-produced, formulated and applied at high inoculum rates in a similar fashion as chemicals for the management of weeds are termed as **bioherbicides**. Although a variety of microbial agents may be used, host specific fungal pathogens have been studied more extensively for biocontrol of weeds; therefore, the term mycoherbicide has often been used interchangeably with bioherbicide (Watson, 1989).

The fungal kingdom offers enormous biodiversity with 1,00,000 known species and over 10,000 fungal species are known to cause diseases on plants (Agrios, 2005, Kirk *et al,*. 2008). It is perhaps surprising, considering awareness of the potential of plant diseases to cause crop failures (e.g. late blight of potato by *Phytophthora infestans*, blast of rice by *Pyricularia oryzae*, coffee rust by *Hemileia vastatrix*) for a century or more, that scientists had overlooked the extent to which wild plants are also vulnerable to fungal infections, thus presenting an opportunity to turn fungal diseases to the advantage of the farmers as weed killers (Aneja, 1999).

Despite the interest in this area of weed control, there are few commercially developed bioherbicides. There has been a great number of naturally occurring fungal strains researched for possible use as mycoherbicides, but only a small proportion have been developed to commercial products. The challenges that have limited the advancement of bioherbicides have been categorized into four constraints: (i) biological factors; (ii) environmental factors; (iii) technological factors; and (iv) commercial factors (Boyetehko and Peng, 2004). For foliar pathogens, biological and environmental factors: temperature, free moisture (lengthy dew requirements) and protection from UV irradiation, all three critical for infection; inconsistent efficacy and limited host range. For any mycoherbicide to

be successful, the host-pathogen balance must be tipped to favour the pathogen.

FUNGAL BIODIVERSITY

Biological diversity or **biodiversity** in simple words is the total variability of life on Earth. Biodiversity is not simply the total number of species, it encompasses the complexity, richness and abundance of nature at all levels from the genes carried by local populations to the layout of communities and ecosystems across the landscape and the value of life on earth.

As per the latest biodiversity estimate there are 8.7 million eukaryotic species on our planet, of which 12,33,500 have been described, it means that a staggering 86% of land species and 91% of marine species remain undiscovered (Mora *et al.*, 2011).

There are no accurate estimates of the total number of fungal species on a global or regional basis. The distinguished tropical botanist, E. J. H. Corner said that 'there are as many species of fungus as there are species of flowering plants if not of all seed plants, multiplied by the number of their parts. The number of species so far discovered, however, is probably only a small proportion of these that exist, as few habitats and regions have been intensively studied (Subramanian, 2013). Hawksworth's estimate of 1.5 million species remains the most widely accepted view (Hawksworth, 1991). It has been calculated that 1200 species are discovered each year. Amongst the over 3,38,000 names at species rank proposed for fungi, 1,00,000 fungal species have been accepted by the scientific community. To date, there are more than 10,000 phytopathogenic fungal species known to cause diseases on plants, compared with 50 species that cause diseases in humans (Agrios, 2005; Kirk *et al.*, 2008).

Phytopathogenic fungi on the basis of their mode of nutrition are classified into three forms: **necrotrophs** or **perthotrophs** (derive their energy from killed cells); **biotrophs** (derive their energy from living cells, mainly through haustoria); and **hemibiotrophs** (the organism having an initial biotrophic phase followed by a necrotrophic phase).

Many potential mycoherbicides have been found to be hemibiotrophs, in which the biotrophic phase provides high host specificity and the necrotrophic phase causes extensive tissue death (Goodwin, 2001).

BIOLOGICAL WEED CONTROL STRATEGIES

Biological weed control with fungal plant pathogens is approached from one of two strategies depending upon the pathogen discovered: the classical strategy and mycoherbicide strategy (Daniel *et al.*, 1973; Templeton *et al.*, 1979). In the **classical strategy**, a fungus is simply introduced or released into a weed population to establish, in time, an epiphytotic requiring no further manipulation. In a severe epidemic, the weed is killed or stressed such that its population is reduced to economically acceptable levels. Pathogens with a low level of virulence are frequent, may co-exist stable with their host pathogens with intermediate pathogenicity, and are good candidates for the classical strategy, maintaining a stable interaction and efficiency. The probability of extinction of a pathogen increases when pathogenicity is greater than a critical value at the intermediate range. The pathogens used in this strategy are generally rusts and other fungi capable of self-dissemination through air borne spores.

Inundative (or mycoherbicidal) strategy- Danial *et al.* (1973) were the first to introduce the mycoherbicide concept. They demonstrated that endemic fungal pathogens might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particularly susceptible stage of weed growth. Since this initial definition of the concept, the term mycohericide has been redefined. Mycoherbicides are simply plant pathogenic fungi developed and used in the inundative strategy to control weeds the way chemical herbicides are used. They are highly specific disease-inducing fungi which are isolated from weeds, cultivated in fermentation tanks and sprayed in fields to control biologically a specific weed without harm to the crop or any non-target species in the environment (TeBeest and Templeton, 1985; Templeton *et al.*, 1988). The pathogens with high levels of virulence may exist in nature in low frequencies due to high extinction rates and are suitable for mycoherbicidal (or inundative) strategy (Yang and TeBeest, 1992).

A third approach – the **manipulated mycoherbicide strategy** – was suggested by Sands and Miller (1993). In this strategy, lethal broad host-range pathogens are genetically modified to permit their safe release. Either they are rendered host-specific or they are given a chemical dependency that prevents their spread or long term survival. The genetic- manipulative approach offers numerous and diverse scenarios for biocontrol of weeds and may open the door to large-scale corporate development and perhaps also to larger-scale public development.

STATUS OF MYCOHERBICIDES

Several recent reviews have provided an overview on various bioherbicide projects being conducted around the globe (Charudattan, 2001; Aneja, 2009; Ash, 2010; Bailey *et al.*, 2010; Aneja *et al.,* 2013). Currently, a total of 17 mycoherbicides (8 in the USA, 4 in Canada, 2 in South Africa, and 1 each in the Netherlands, Japan, and China) have been registered around the globe (**Table 1**). In addition to the commercialized products, Charudattan (2001) also listed over 50 examples of pathogen-weed combinations which have been reported worldwide as having potential as bioherbicides. A further search of the literature using the ISI Web of Science (http://apps isiknowledge.com) database has revealed 509 papers

Table 1. Examples of mycoherbicide agents at various stages of development and commercialization.

Countries and registeration year	Pathogens and commercial name	Target weeds	Formulation types and its constituents
China, 1963	**Lubao**, *Colletotrichum gloeosporioides* f.sp. *cuscutae*	Dodder (*Cuscuta* spp.) in soybeans	**Liquid**: Conidial suspension
USA, 1981	**DeVine®**, *Phytophthora palmivora*	Strangler vine (*Morrenia odorata*) in citrus orchards	**Liquid**: Chlamydospores in water
USA, 1982	**Collego™ (LockDown™)**, *Colletotrichum gloeosporioides* f.sp. *aeschynomene*	Northern joint vetch (*Aeschynomene virginica*) in rice & soybeans	**Liquid**: Wettable powder component A (dried spores) and component B (rehydrating agent + surfactant)
USA, 1983	**Casst™**, *Alternaria cassiae*	Sickle pod & coffee senna (*Cassia* spp.) in soybeans & peanuts	**Solid**: Water + nonoxynol surfactant (0.04%); paraffin wax, mineral oil, soyabean oil, corn syrup,lacithin
USA, 1984	**ABG-5003,** *Cercospora rodmanii*	Waterhyacinth (*Eichhornia crassipes*) in Florida waterways	**Wettable powder**; Mycelial fragments and conidi
USA, 1987	**Dr BioSedge**, *Puccinia canaliculata*	Yellow nutsedge (*Cyperus esculentus*) in soybeans, sugarcane, maize, potato & cotton	**Emulsified suspension**
Canada, 1992	**BioMal**, *Colletotrichum gloeosporioides* f.sp. *malvae*	Round-leaved mallow (*Malva pusilla*) in wheat, lentils & flax	**Solid**: Wettable powder in silica gel carrier
USA, 1993	**PESTA**, *Colletotrichum truncatum*	Hemp sesbania (*Sesbania exaltata*)	**Liquid**: Water + nonoxynol surfactant (0.02%); paraffin wax, mineral oil, soybean oil, lecithin
USA, 1993	**PESTA**, *Fusarium oxysporum*	*Cassia* spp.	**Solid**: Fungus-infested wheat gluten
South Africa, 1997	**Stumpout™**, *Cylindrobasidium laeve*	*Acacia* species in native vegetation & water supplies	**Liquid**: Oil suspension
Netherlands, 1997	**BioChon™**, *Chondrostereum purpureum*	Woody weeds, e.g. black cherry (*Prunus serotina*) in plantation forests	**Liquid**: Mycelial suspension in water
South Africa, 1999	**Hakatak**, *Colletotrichum acutatum*	*Hakea gummosis* & *H. sericea* in native vegetation	**Liquid**: Conidial suspension
USA, 2002	**Woad Warrior**, *Puccinia thlaspeos*	Dyers woad (*Isastis tinctoria*) in farms, rangeland, waste areas, & roadsides	**Solid**: Powder
Canada, 2004	**Chontrol™ = Ecoclear™**,*Chondrostereum purpureum*	Alders, aspen & other hard-woods in right	**Liquid**: Spray emulsion and spray
Canada, 2004	**Myco-Tech™ paste**, *Chondrostereum purpureum*	Deciduous tree species in rights of way & forests	Liquid: Paste
USA, 2005	**Smolder®**, *Alternaria destruens*	Dodder species in agriculture, dry bogs & ornamental nurseries	**Liquid**: Conidial suspensionTwo types:Smolder G- soil applied granularSmolder WP- conidial suspension spray formulation
Canada, 2007	**Sarritor**, *Sclerotinia minor*	Dandelion (*Taraxacum officinale*) in lawns/turfs	**Granular**
Africa, 2008	**Striga**, *Fusarium oxysporum* f.sp. *strigae*	*Striga hermonthica* and *S. asiatica*	**Solid:** Dried chlamydospores + Arabic gum
India, 2014	**Gibbatrianth***, *Gibbago trianthemae*	Horse purslane (*Trianthema portulacastrum*) in pigeon pea, soybean, potato, maize, sorghum	**Liquid**: Conidial suspension + surfactant

***In search of collaborator for commercialization**

published which mentioned bioherbicides or mycoherbicides since 1987 clearly indicating the plethora of articles on "potential bioherbicides". The consensus is that until an agent is commercialized it should be called a biological control agent and the term bioherbicide or mycoherbicide should be reserved for the formulated, marketed and commercialized product.

Essentially the discovery and development of bioherbicides has been undertaken by plant pathologists, weed scientists or entomologists and begins with the observation of naturally occurring diseased plants. The early stages of the investigation follow traditional plant pathology techniques including the application of Koch's postulates and other standard techniques taught in undergraduate and postgraduate plant pathology (Agrios, 2005; Aneja, 2003; 2014). At this stage high concentrations of single or mixed inoculum are used to increase the likelihood of plant mortality. Following the isolation of a likely candidate, the research programme often includes; culture purification, host range testing, laboratory scale fermentation and preliminary formulation (**Fig. 1**).

Out of the several bioherbicides developed around the globe, a few of them are commercially available (e.g. DeVine, Stumpout, Chontrol, EcoClear, Mycotech, Sarritor). The others are unavailable due to lack of continued commercial lacking, high cost of mass production, and resistance in some weed biotypes (eg. Dr Biosedge), low sales or regulatory concerns and lack of funding for infrastructure, personal and patenty of the product; close collaboration between non-industrial and industrial sectors for commercialization. One BCA, *Colletotrichum gloeosporioides* f.sp. *aeschynomene* has been re-registered (previously Collego) as of March 2006 under the commercial name LockDown for use in rice in the three states of USA; Arkansas, Lousiana and Mississippi (Yandoc *et al*., 2006). A number of reviews have offered reasons why bioherbicides developed have not been successful (Auld and Morin, 1995; Weston, 1999; Aneja, 1999; 2009; Hallett, 2005).

Many potential biological control agents of weeds have been found to be hemibiotrophs. Hemibiotrophs have an initial biotrophic phase followed by a necrotrophic phase (Bailey *et al.,* 1992). This combination can result in both relatively high specificity and virulence (i.e. the degree of pathogenicity). The biotrophic phase is considered to provide high host specificity and the subsequent period of necrotrophic phase causes extensive tissue damage or death of the weed. *Colletotrichum* spp. share both these characteristics, hence are commonly found on lists of promising mycoherbicides (Templeton 1992; Watson *et al*., 2000; Goodwin, 2001; Aneja, 2009).

Literature search (Charudattan, 2001; Ash, 2010; Aneja *et al.*, 2013) reveals that much of the research in bioherbicides development is specific to a single pathogen/host system, the way ahead in bioherbicide research would appear to be the development of bioherbicide based on several host-specific fungal pathogens in a bioherbicide mixture as a multiple-pathogen

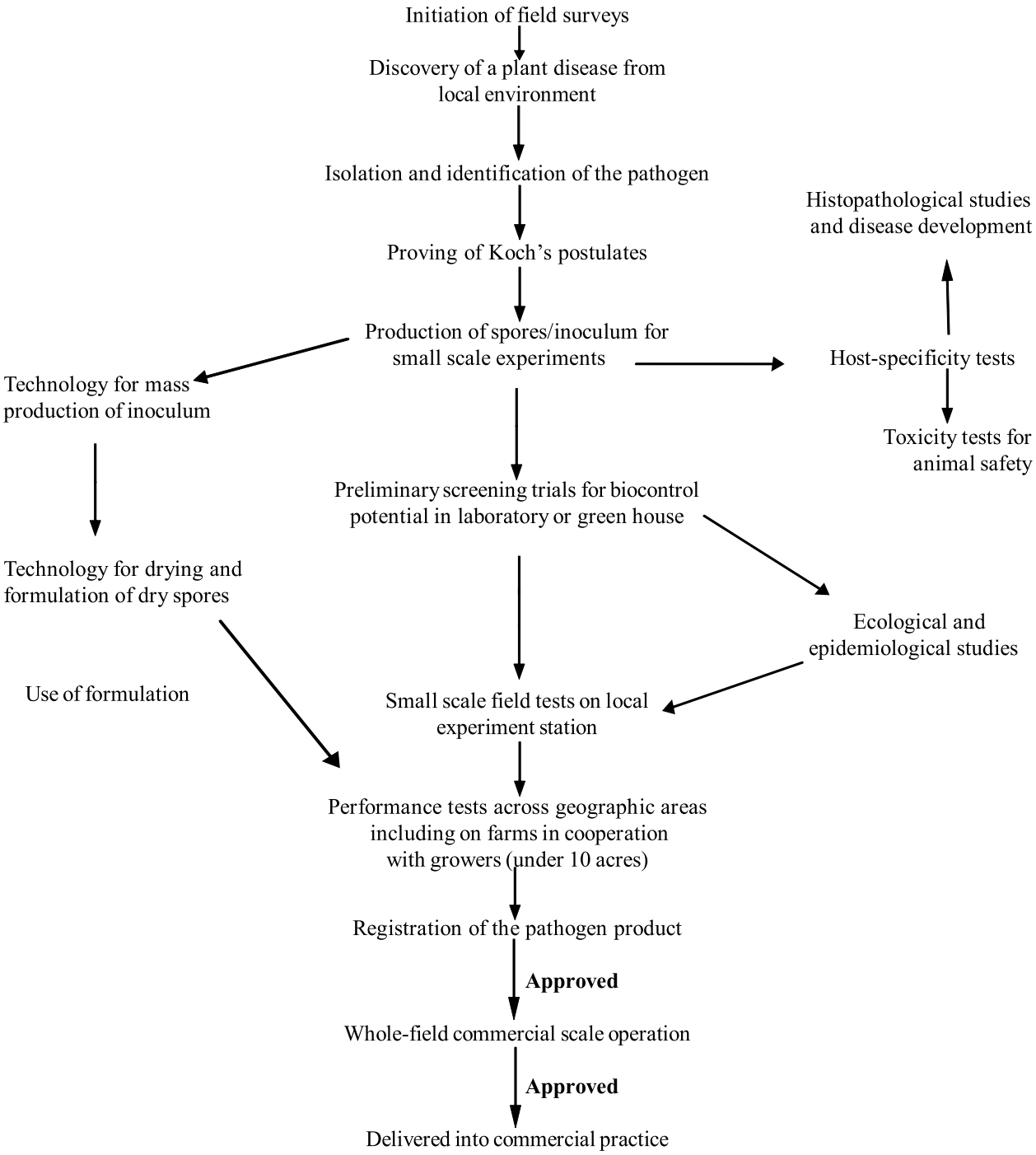

Fig. 1. Protocol for the development of a fungal biocontrol agent into a mycoherbicide (Adapted from Aneja, 1999).

system for simultaneous control of several weeds (Chandramohan and Charudattan, 2003; Ash, 2010). Several weed biocontrol agents are not sufficiently virulent for field release. Since hydrolytic enzymes play an important role in the pathogenicity of plants by facilitating fungal penetration through the host cell wall. Current research is being carried out by adding the exogenous cellulase and /or pectinase with the mycoherbicidal agent to accelerate fungus infection thereby enhancing the weed control by a fungus (Babalola, 2007; 2010).

BIOCONTROL WORK CARRIED OUT AT KURUKSHETRA

During the last 30 years, searches for fungal biocontrol agents (BCAs) have been made on 26 weeds, both aquatic and terrestrial, in northern India (Haryana, Punjab, Himachal Pradesh, Delhi and U.P.). Of the various fungal pathogens identified on different weeds, several of them have been reported for first time on the notorious weeds of this region such as water hyacinth, horse purslane and parthenium (**Table 2**). The various host-pathogen systems

Table 2. Fungal pathogens recorded on *Eichhornia crassipes, Trianthema portulacastrum* and *Parthenium hysterophorus* at Kurukshetra.

S. No.	Fungal pathogen	Symptoms
Eichhornia crassipes		
1.	*Fusarium* sp.	Leaf decay
2.	*Fusarium equiseti*	Leaf spot
3.	*Cercospora piaropi*	Leaf spots and leaf necrosis
4.	*Marasmiellus inoderma*	Foliar blight
5.	*Cephalosporium eichhorniae (Acremonium zonatum)*	Stem and root rot
6.	*Cylindrocladium scoparium* var. *brasiliensis*	Leaf spot
7.	*Helminthosporium bicolour*	Leaf spot
8.	*Alternaria eichhorniae*	Leaf spot and severe leaf blight
9.	*Myrothecium roridum*	Leaf spot
10.	*Cercospora rodmanii*	Leaf spot
11.	*Curvularia lunata*	Leaf spot
12.	*Fusarium solani*	Raddish brown leaf spot
13.	*Rhizoctonia solani*	Foliar blight
14.	*Alternaria alternata*	Leaf spot
15.	*Fusarium chlamydosporum*	Leaf spot
16.	*Epicoccum nigrum*	Leaf spot
17.	*Phoma sorghina*	Leaf spot
18.	*Bipolaris sorokiniana*	Leaf spot
19.	*Alternaria alternata* (AL-14)	Leaf spot and leaf blotches
Trianthema portulacastrum		
1.	*Gibbago trianthemae*	Leaf spot
2.	*Fusarium chlamydosporum*	Leaf spot
Parthenium hysterophorus		
1.	*Cladosporium cladosporioides*	Leaf spot
2.	*Alternaria alternata*	Leaf spot
3.	*Erysiphe cichoraceraum*	Powdery mildew
4.	*Curvularia lunata*	Leaf spot
5.	*Pseudocercospora* sp.	Leaf spot
6.	*Cercospora partheniiphila*	Leaf spot
7.	*Myrothecium* sp.	Leaf spot
8.	*Fusarium* sp.	Leaf spot
9.	*Colletotrichum* sp.	Anthracnose
10.	*Alternaria zinniae*	Leaf spot

For references see Aneja, 1996; 1998; Aneja *et al*., 2014a;b.

for their evaluation as potential biocontrol agents are described below.

GIBBAGO TRIANTHEMAE – HORSE PURSLANE SYSTEM

Horse purslane (*Trianthema portulacastrum*, Family *Aizoaceae*), is an introduced terrestrial weed in India. It has become a noxious weed due to competition for yield in various agricultural and vegetables crops such as mustard, maize, pigeon pea, soybean, potato and onion in northern India. Up to 60-70% infestation of this weed has been reported in pigeon pea and soybean fields and 80-90% in maize and brassica fields. Between 1989 and 1998 a series of surveys of plant pathogenic fungi associated with naturally infected horse purslane were conducted in the states of Haryana and Punjab. Infected leaves (**Fig. 2**) collected from various sites, yielded a species of *Gibbago*, identified as *Gibbago trianthemae*

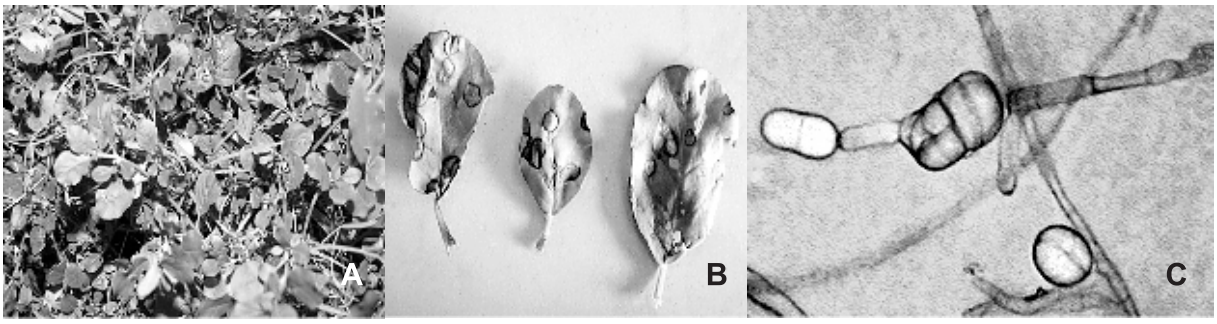

Fig. 2. (A) *Trianthema portulacastrum* infected plants; (B) Leaf spots due to *Gibbago trianthemae;* (C) Germinating conidia of *G. trianthemae*.

Simmons, a phaeodictyoconidial hyphomycetous fungus reported on horse purslane for the first time in India (Aneja and Kaushal, 1998). In experimental pots, defoliation started after 20 days of inoculum spraying. Per cent infection on leaves ranged between 72 and 84 per cent, 30 days post inoculation with a conidial suspension at concentration of $2.2x10^5$ conidia/ml. Application of inoculum significantly reduced the production of leaves, height and biomass per plant as compared to control. Germination of conidia (**Fig. 2**) took place within 6 to 12 hours. Symptoms on leaves initiated as small pin point lesions, 3-4 days after spraying of inoculum. A significant correlation between the growth and sporulation of the pathogen was reported when tested on 10 different culture media. Best sporulation was found on trianthema extract dextrose agar followed by potato dextrose agar and potato dextrose agar+yeast extract ($8.6x10^5$> $8.0x10^5$> $7.37x10^5$ conidia/ml, respectively). Fungus showed growth but failed to sporulate in all the three broths. Best sporulation was recorded at 25 °C. Conidia germinated between 15 and 35 °C, the best recorded at 25 °C. Biocontrol studies conducted on the *Trianthema-Gibbago* system revealed that *G. trianthemae* has most of the criteria desirable for development it as a mycoherbicide to control horse purslane; *i.e.* it can be cultured on a cheap medium (trianthema extract dextrose agar), good sporulation capacity, host-specificity, fast growth rate and hence can be mass produced in a short time, and infection can take place from conidia and/or mycelial fragments (Aneja *et al.,* 2000; Aneja, 2010). The formulation of the fungus + surfactant has been named Gibbatrianth (**Table 1**).

ALTERNARIA ALTERNATA-WATER HYACINTH SYSTEM

During the series of surveys conducted between 1985-87 in Haryana, a leaf spot disease severely affecting the water hyacinth (*Eichhornia crassipes*) population on a pond (1.5 ha) at Kurukshetra was observed. Symptoms on the leaves showed lesions of various sizes ranging from minute dark brown lesions to large irregular patches (**Fig. 3**). The disease was regularly observed at Kurukshetra between 1985 and 1987 severely damaging this weed. On the basis of symptomology, cultural and microscopic characteristics (beaked, muriform, dark, catenate conidia arranged in acropetal manner) the pathogen was identified as *Alternaria alternata* (**Fig. 3**). Water hyacinth leaves responded differently towards infection at different growth stages and under different conditions in the experimental pits. Infection in covered pits which were aseptically sprayed with inoculum of the pathogen was less than the uncovered pits. Disease incidence showed an increasing trend from small medium to large water hyacinth leaves in the field as well as pits. This pathogen on evaluation for its biocontrol potential in the experimental pits showed host specificity and killing of the host alone and in combination with insect *Neochaetina eichhorniae* (Aneja and Singh, 1989; Aneja, 1998). The pathogen has shown potential to be developed as a mycoherbicide.

ALTERNARIA EICHHORNIAE-WATER HYACINTH SYSTEM

Alternaria eichhorniae was first described in 1970 from Bangalore and Assam, India, as the causal agent of leaf-

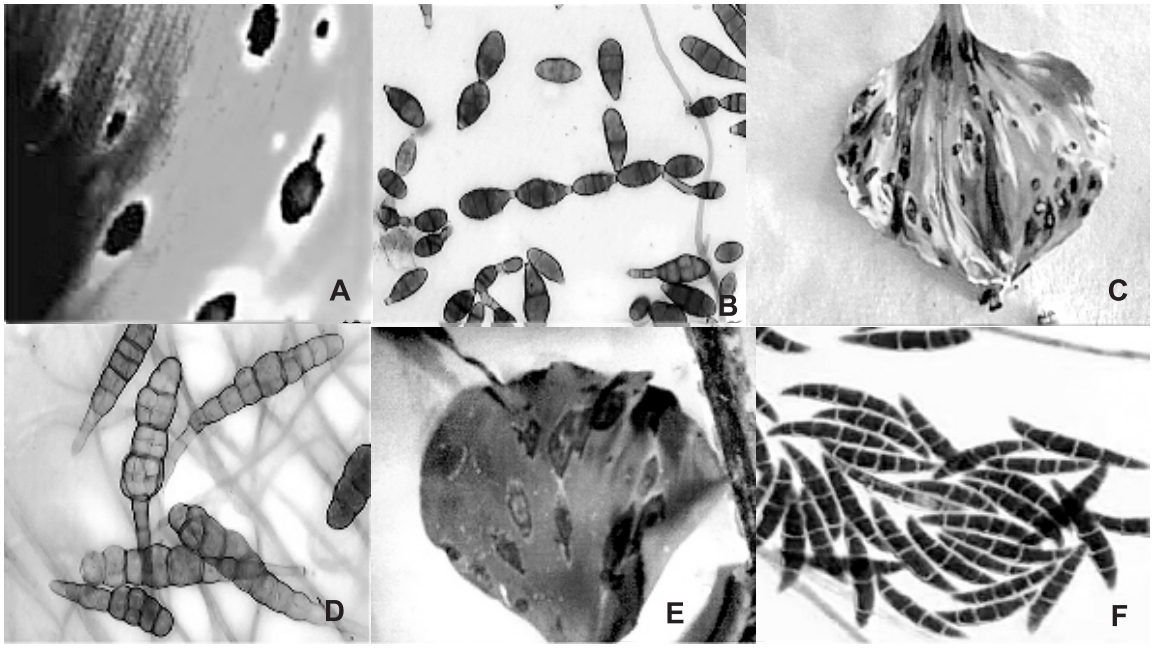

Fig. 3. Disease symptoms and conidial characteristics of three potential fungal biocontrol agents of water hyacinth: *Alternaria alternata* (A&B), *A. eichhorniae* (C&D), *Fusarium chlamydosporium* (E&F). (A) Leaf spots. (B) Small beaked, muriform, pigmented, catenate conidia in acropetal manner. (C) Leaf spots. (D) Long beaked muriform pigmented conidia. (E) Leaf spots. (F) Hyaline, sickle shaped, transversely septate macroconidia produced in a sporodochium.

spot disease of water hyacinth and was suggested as a suitable biocontrol agent of this weed (Nag Raj and Ponnappa, 1970). During the surveys conducted between 1990 and 1992 in Haryana, a new leaf spot disease was observed on water hyacinth at Kurukshetra. Symptoms on the leaves were observed as discrete or blotchy necrotic leaf spots with dark centres and brownish black margins, often with a thin yellow halo surrounding the spots (**Fig. 3**). On the basis of morphological and cultural characteristics, this pathogen was identified as *Alternaria eichhorniae*. This pathogen was evaluated for its biocontrol potential in experimental pits at Kurukshetra, and caused infection up to 80% on leaves. Older leaves were found to be more susceptible to the pathogen. Uncovered pits showed more infection in comparison of covered pits. The disease development was more rapid at 25 °C (Aneja, 1996; 1998). Other features of potential mycoherbicide were also present in this pathogen.

FUSARIUM CHLAMYDOSPORUM-WATER HYACINTH SYSTEM

Another leaf spot disease caused by *Fusarium chlamydosporum* (**Fig. 3**) was recorded from Kurukshetra. Macroconidia of this fungus are sickle shaped with a narrowly rounded to pointed apex. The infection of water hyacinth leaves after one month post inoculation with conidia of *F. chlamydosporum* ranged between 25 and 54%. In covered pits infection was lower than the uncovered pits. Green house and laboratory experiment results revealed that large sized leaves exhibited more infection than the small and medium sized leaves (Aneja *et al.,* 1990; Aneja, 1998).

FUTURE OUTLOOK

The pace of development in the area of commercialisation of mycoherbicides is still slow because of an array of biological, economic and regulatory constraints. To overcome biological constraints- such as virulence, stability, producing sufficient concentration of spores to be economically viable, host range, environmental conditions, dew requirement of the pathogen and geographic biotypes of the weeds- the use of fungal biotechnology by genetic manipulation of fungal pathogens through protoplast fusion, improvement of the fermenters, spraying techniques, and modification of the carriers to the inoculum is being currently pursued in order to enhance the development of mycoherbicides into products.

In spite of better potential of fungi to control obnoxious weeds in the tropical climate than in temperate countries, researchers in the Indian subcontinent are still in the pioneering stages of using fungi as biocontrol agents of weeds. Considering the rich biodiversity and good prospects for discovering and developing biocontrol agents for many types of weeds, progress made in this area in the developing countries has been almost nil. It seems the major constraint is the lack of availability of funds. For example, for the development of potential biocontrol agents into a mycoherbicide, the first step is its identification. Scientists in developing countries do not have enough funds to pay for authentic identification and unless the identification is confirmed, it is not possible to go ahead with publication and further evaluation for efficacy and development and commercialisation of the agent. Moreover, private enterprises in developing countries are unwilling to give assistance for any research projects unless they are guaranteed one hundred per cent economic returns on their investment.

Intensive and long term research is needed for finding out and understanding the unique biology of specific biocontrol agents, target host-pathogen combinations, environment impact and their potential in the management of weed. The future of a mycoherbicides appears to be promising, if proper and immediate attention is paid, especially in the developing countries.

- Conducting systemic field surveys for the identification of the major endemic diseases of the major weeds.
- Isolation and establishment of stable culture of the causal organisms.
- Selection of pathogens, particularly those which are highly pathogenic.
- Developing methods for mass production of the stable inoculum.
- Understanding the disease cycle and the weed pathogen system.
- Understanding the genetics of the pathogen or molecular basis of the disease that may eventually help in selecting and establishing pathogens with greater virulence (super pathogens).
- To appreciate fully the value of mycoherbicides the farmers must be taught the limitations and benefits of the new technologies prior to or during market launch of any new bioherbicide.
- The application of several host-specific fungal pathogens in a bioherbicide mixture as a multicomponent bioherbicide system for simultaneous, broad-spectrum weed biocontrol.
- Enhancement of mycoherbicidal activity of BCAs by the application of exogenous cellulase and/or pectinase enzymes.

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