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Biological Control of Weeds with Pathogens: Current status and Future trends

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Abstract

Considering the implementation of biological control as a modern weed control trend depends primarily on several strategies, most prominently is searching for alternatives to chemical control methods aimed to minimize hazards resulting from herbicide residue on both human and animal health, and on the ecosystem in general. In addition, one of the major strategies of the biological control concept is attempting to incorporate the biological weed control methods as a component of integrated weed management to achieve satisfactory control results and meanwhile, reduce herbicide application to the minimum extent possible. Many pathogens with mycoherbicide potential have been discovered, but few have become commercial realities or viable alternatives. Biological, technological, and commercial constraints have hindered progress. Many of these constraints are being addressed, but there is a critical need to better understanding the biochemical and physiological aspects of pathogenesis of potential mycoherbicides. Weak links in the host plant's defense need to be exploited and the virulence of pathogens enhanced. In order to make a significant jump forward in formulation, applied research must be evaluated to include fundamental studies of physiological and biochemical changes in cellular organelles and membranes as affected by desiccation and by protections against desiccation. Shelf-life data are worth very little in practical terms for microbial products without data on bioassays and on tolerance to environmental extremes. Environmental Tolerance studies and bioassays are essen-tial for monitoring any changes in process. Toxic metabolites produced by fungal pathogens play an important role in host-pathogen interactions. These metabolites consist of a wide array of chemical structures. They can be important factors of pathogenicity or virulence, can have different behaviors' with respect to the host varying from strictly host-specific to completely non-specific compounds, and can act with different mechanisms affecting several sites in the host.

Key-Words: Biological control; formulation; genetic engineering; herbicides, integrated weed management; mycoherbicides; phytotoxins; weed control

Introduction

Chemical herbicides are presently the most effective immediate solution to most weed problems. However, the high costs involved in developing and registering chemical herbicides, along with environmental problems concerning pesticides in general have prompted researchers to investigate alternative systems of weed control. Ideally, such systems should control weeds to the same extent as chemical herbicides without posing a threat neither to the environment nor to non-target species (Auld and Morin 1995; Boyette et al. 1996).\

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It is becoming widely accepted, that a sole dependence of weed control strategies on chemical herbicides is inopportune and that alternative and complementary control options should be considered. Hence, the overall intention of new control strategies is to suppress problematic weed populations in an ecologically and economically feasible manner by integrating a number of different methods. Biological weed control represents an eligible strategy that can be integrated into such a system. Thereby biocontrol methods should be considered as components of an overall integrated weed management in order to achieve efficiency and to reduce herbicide application to the minimum possible extent.

The biological control of weeds by plant pathogens has gained acceptance as a practical, safe, and environmentally beneficial method applicable to agro-ecosystem. The application of plant pathogens comes

especially into consideration for parasitic weeds, for weeds difficult to control via chemical means, or for small-scale and specialized crops where the development of specific chemical solutions is too expensive (Schroeder et al. 1993; Auld and Morin 1995). Other instances where plant pathogens may be preferable over chemical herbicides are aquatic weeds to avoid chemical contamination of water and situations where a close relation between weeds and crops account for a high degree of specificity such as grass weeds in cereal production (Auld and Morin 1995).

2 What is the strategy of biological weed control?

Biological weed control is the deliberate use of natural enemies to suppress the growth of a weed or to reduce a weed population (Watson 1989). There are two basic strategies to implement the biological control of weeds by pathogens. The introduction of foreign pathogenic organisms, often called the 'classical approach', and the 'augmentative' or 'bioherbicidal approach', where the pathogenic organ-isms are already present (native or introduced) and their population is increased by mass rearing. In epidemiological terms, these approaches are also often described as 'inoculative' and 'inundative strategy' (Charudattan and de Loach 1988; Hasan and Ayres 1990; Watson and Wymore 1990; Mueller-Schaerer and Frantzen 1996; Mueller-Schaerer and Scheepens 1997).

The 'inoculative' or 'classical approach' implies the control of invasive weeds by introducing nonnative control organisms from the weed's natural habitat. These pathogens are released only on a small part of the total infested area and the control is achieved by gradual spread of the initial population.

At this, a successful control strongly depends on favorable conditions promoting an effective increase in the population of the controlling organism, and the establishment of epiphytotics to reduce the target weed population (Mueller-Schaerer and Scheepens 1997). The 'inundative' or 'bioherbicides' strategy uses periodic releases of an overabundant supply of the controlling organism to suppress the entire weed population. Such pathogens or biological agents are generally "manufactured", formulated, standardised, packed and registered like chemical herbicides (Auld and Morin 1995; Mueller-Schaerer and Scheepens 1997). One group of such biological agents with promising potential for weed control are mycoherbicides.

3 What's the tactic of mycoherbicide?

Mycoherbicides have been 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) or as "living products that control specific weeds in agriculture as effectively as chemicals" (Templeton et al. 1986). Mycoherbicides are specifically formulated preparations of a living inoculum of a plant pathogen that is used for the control of a target weed. Usually they are applied in a manner similar to chemical herbicides by periodic dispersals of distinct doses of the virulent inoculum (Watson 1989; Watson and Wymore 1990). The concept of mycoherbicides was first introduced by Daniel et al. (1973), who demonstrated that an endemic pathogen might be rendered completely destructive to its weedy host by applying a massive dose of inoculum at a particularly susceptible growth stage. The application of an inundative dose of inoculum and its proper timing shortens the lag period for inoculum build-up and pathogen distribution, essential for natural epiphytotics. To render this ap-proach a success, the pathogen must be culturable in artificial media; the inoculum must be capable of abundant production using conventional methods such as liquid fermentation; the final product must be genetically stable and specific to the target weed; storage (shelf-life), handling, and methods of application must be compatible with current agricultural practices; and the pathogen must be effica-cious under sufficient different environment conditions to allow a feasible application window (Dan-iel et al. 1973; Templeton et al. 1979).

4 Mycoherbicide candidates of important weeds

The level of scientific activity in mycoherbicide research has increased tremendously since the early eighties of the last century. Both the number of weeds targeted for control and candidate pathogens studied have increased. Practical registered or unregistered uses of mycoherbicides have also increased worldwide. Likewise, the numbers of U.S. patents issued for mycoherbicidal use of fungi and myco-herbicidal technology have increased, perhaps foretelling an increased reliance on mycoherbicides in the future. Presently two mycoherbicides, DeVine® and Collego®, are used commercially in the United States to control, respectively, milkweed vine, *Morrenia odorata*, in citrus groves of Florida and northern joint vetch, *Aeschynomene virginica*, in rice and soybean fields of Arkansas and neighboring states (Templeton and Heiny 1988; Charudattan 1991; TeBeest et al. 1992).DeVine, marketed by Abbott Laboratories, is the first registered mycoherbicide.

The mycoherbicidal product consists of a liquid concentrate of chlamydospores of pathotype of *Phytophthora palmivora* with a shelf life of six weeks in refrigerated storage (Woodhead 1981; Kenney 1986;

Ridings 1986). Collego is applied post-emergence, aerially or with land-based sprayers. It is marketed as a dry formulation consisting of 15 % viable, dry conidia of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and 85 % inter ingredients. The history, development, registration, integrated use, and post-registra-tion status of Collego have been reviewed (Klerk et al. 1985; TeBeest and Templeton 1985; Smith 1986; Templeton 1986).

The mycoherbicide BioMal® *(Colletotrichum gloeosporioides* f. sp. *malvae)* has been registered in Canada and is used against round-leaf mallow, *Malva pusilla* (Auld and Morin 1995; Goodwin 2001). For control of sicklepod *(Cassia obtusifolia),* "CASST" is formulated as spores of *Alternaria cassiae* in emulsifiable parafinic oil (Boyette et al. 1996).

Several other candidates have undergone extensive testing for commercial development. These include *Colletotrichum orbiculare* for spiny cocklebur, *Xanthium spinosum* (McRae and Auld 1988; McRae et al. 1988; Auld 1993); *Sclerotinia sclerotiorum* for Canada thistle, *Cirsium arvense* (Brosten and Sands 1986); *Colletotrichum coccodes* for velvet leaf, *Abutilon theophrasti* (Wymore and Watson 1989); *Colletotrichum malvarum* for prickly sida, *Sida spinosa* (Kirkpatrick et al. 1982); *Fusarium solani* f. sp. *cucurbitae* for Texas gourd, *Cucurbita texana* (Weidmann 1988; Weidmann and Templeton 1988); *Lasiodiplodia theobromae* for *Parthenium hysterophorus* (Kumar and Singh 2000); *Fusarium oxysporum* for the narcotic plant coca, *Erythroxylum coca* (Gracia-Garza and Fravel 1998); and *Phomopsis convolvulus* for field bindweed, *Convolvulus arvensis* (Ormeno-Nunez et al. 1988; Morin et al. 1989 and 1990; Vogelgsang et al. 1994 and 1998; El-Sayed and Hurle 2001). Many other pathogens are under various stages of research and development.

5 Technological constraints of mycoherbicides

The type of biocontrol agent, whether classical or augmentative, dictates the criteria for the development of appropriate formulation and application technology. For classical control, cost may not be as important, because long-term economic benefit will be realized if the agent is successful. Furthermore, optimization of control in the application area is not necessarily required in the first year, because successful agents will be self-perpetuating. The formulation requirements for classical agents are more flexible, because the applications will most likely be performed by someone trained in application techniques for biological weed control. For augmentative control, the goal is to produce a commercially viable, consistently efficacious product. Formulation and application methods must be adaptable to conventional equipment, and have adequate shelf life for marketing purposes. Once the product is commercialized, it will be applied by people who have little or no experience in working with microbes and their unique environmental sensitivities. Therefore, the formulation and application method needs to be as insensitive as possible to environmental fluctuations and reasonable variations in application protocol.

Shelf life is much more important, because the products will be stored for marketing over a long period of time, often in facilities with variable environmental control (Powell and Justsum 1993; Womack and Burge 1993; Auld and Morin 1995; Boyetchko et al. 1998).

6 Formulation of mycoherbicides

An adequate formulation is one of the major technological constraints to the development of reliable and efficacious mycoherbicides (Auld and Morin 1995). The most challenging aspect of formulation of mycoherbicides is to overcome the dew requirement that exists for several mycoherbicides. In addition, appropriate formulations can also reduce the dosage of inoculums required to kill weeds, thus potentially reducing the cost of mycoherbicides (Amsellem et al. 1990).

Recent research on formulation has shown the potential for invert (water-in-oil) emulsions for mycoherbicides (Daigle et al. 1990; Connick et al. 1991). In this type of formulation, spores of the fungus in water droplets occur within a continuous oil phase. Experiments conducted with a number of potential bioherbicides have demonstrated that an invert emulsion allowed infection to occur in the absence of available water and reduced the need to apply high dosages of inoculums (Daigle et al. 1990; Boyette et al. 1993; Yang et al. 1993). However, an invert emulsion may be difficult to apply with conventional equipment because of its viscosity and may cause non-target damage (Auld 1993; mWomack and Burge 1993). An invert emulsion has been shown to cause phytotoxicity in some cases and to predispose a variety of plants to opportunistic pathogens (Amsellem et al. 1991). Studies are currently being conducted to screen a variety of oils and emulsifying agents to improve initial invert emulsion formulations for mycoherbicides (Womack and Burge 1993). An invert emulsion formula-tion exhibiting lower viscosity and greater water-retention properties was developed by Connick et al. (1991). The use of low concentrations of vegetable oils with an emulsifying adjuvant was also found to enhance efficacy of *Colletotrichum orbiculare* in inciting

disease on spiny cocklebur in the absence of dew in controlled environments (Auld 1993).

Encapsulation of microbes in sodium alginate and kaolin clay was first described in 1983 for the fungi *Alternaria macrospora, A. cassiae, Fusarium lateritium, Colletotrichum malvarum* and a *Phyllosticta* sp. (Walker and Connick 1983). Alginate has been used extensively in formulations of biological weed control agents, and also in fungal preparations for biological control of soilborne diseases (Papavizas et al. 1987).

The addition of exogenous nutrients to liquid or granular preparations of a potential mycoherbicide has shown promise in enhancing effectiveness of pathogens or increasing sporulation on the surface of granules. Enhanced efficacy has also been achieved by adding surfactants that improve the physical characteristics of liquid formulations (Boyette et al. 1984; Weidemann 1988). Conversely, 64 % sorbitol and gelatine reduced infection by *Phomopsis convolvulus* on field bindweed (Morin et al. 1990).

The addition of cutinase enzymes into the formulation of bioherbicides has been suggested to assist the pathogen *C. orbiculare* in the breakdown of the target plant's cuticle and hence facilitate penetra-tion (McRae and Stevens 1990).

Furthermore, it was found that components of the matrix of *P. convolvulus* may prove useful in the formulation by improving germinability of spores or shelf life of the future product (Sparace et al. 1991).

The enhancement of disease by *Colletotrichum truncatum* in hemp sesbania *(Sesbania exaltata)* by coinoculating with epiphytic bacteria was studied by Shisler et al. (1991). They identified a number of bacterial isolates that stimulated appressoria formation and enhanced disease symptoms on hemp sesbania. Fernando et al. (1994) reported enhanced efficacy of *Colletotrichum coccodes* on velvetleaf when the fungus was coinoculated with phylloplane bacteria. Application of bacterial pathogens to plants requires special formulation considerations. Because bacteria are not able to penetrate plant cuticles directly, their entry to the plant must be artificially facilitated. This has been done successfully in two ways. The first is through the use of a non-ionic organosilicone surfactant, which lowers the surface tension of aqueous solutions to the point that stomates are penetrated (Zidack et al. 1992). A second form of application for plant-pathogenic bacteria is mechanical wounding. The pathogen *Xanthomonas campestris* pv. *poannua* was applied to golf course greens for control of annual bluegrass in conjunction with mowing (Johnson 1994). The moving wounds the plant, providing a mode of entry for bacteria. This has proven to be an effective control for annual bluegrass, and is being developed for commercial use in Japan (Zidack and Quimby 1998).

7 The challenges for development of mycoherbicide formulations

A number of challenges are encountered in the formulation of biocontrol agents, including good market potential, ease for production and application, adequate product stability and shelf life during transportation as well as in storage and guaranteed propagule viability and efficacy over the long term (Boyetchko et al. 1998). Some reasons why biocontrol agents have met with limited commercial success are difficulty of production, sensitivity to UV light and desiccation, requirements of high humidity for infection, insufficient performance over a wide range of environmental conditions, and lack of appropriate formulation (Powell and Jutsum 1993). Formulations should be used to improve product stability, bioactivity, and delivery (i. e., ability to mix and spray the product) as well as to integrate the biopesticide into a pest management system (Boyette et al. 1996). Other important characteristics of a successful formulation are convenience of use, compatibility with end-user equip-ment and practices, and effectiveness at rates consistent with agricultural practices (Boyetchko et al. 1998). For foliar biocontrol agents, environmental factors that influence plant infection and disease development are temperature, free moisture or dew period, and protection against UV irradiation and desiccation (Boyette et al. 1996; Boyetchko et al. 1998). For soil-applied biocontrol agents, physical and chemical characteristics of soil, moisture, and temperature regimens, as well as microbial competi-tion can all influence efficacies (Boyetchko et al. 1998). The inclusion of novel synergists in bioherbicide formulations could take them past the point of research, and into the development of efficacious, reliable, and economical products for the marketplace (Hoagland 1996). All of these parameters need to be taken into consideration when developing an appropriate mycoherbicide formulation.

8 Interactions between chemical herbicides and mycoherbicides

There is increasing interest worldwide in the possible use of mycoherbicide in conjunction with chemical herbicides. It is known that interactions between pathogens and chemical herbicides can result in increases or decreases in disease levels (Altman and Campbell 1977; Charudattan and de Loach 1988; Hoagland 1996). Interaction between herbicides and plant pathogens, herbicide induction of microbial invasion of plant roots, and the interactions of sublethal

herbicide doses on root pathogens have been reviewed (Greaves and Sargent 1986; Smith 1991; Levesque and Rahe 1992). Although most of the examples in the literature deal with herbicide effects on crop plants, the similar disease-increasing ability of herbicides affects weeds as well. Such interactive effects may be useful in suppressing weed growth, reducing weed competition, and improving the efficacy of microbial herbi-cide (Charudattan and de Loach 1988; Hoagland 1996; Zidack and Quimby 1998).

Herbicide-pathogen interactions may occur in the following ways:

- A herbicide that is effective by itself on a weed may nevertheless render it more or highly susceptible to a pathogen.
- A herbicide that is lethal to a weed, when used at sub lethal rates, may improve the weed control efficacy of a pathogen.
- A herbicide or herbicides may be used together with microbial herbicide to increase the spectrum of weeds controlled in a field.
- A herbicide may interfere with and reduce the amount of control that could be obtained with a pathogen alone.

The herbicides acifluorfen and bentazon [3-(1-methyl-ethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] were the most effective synergists, providing increased control of the following weedy hosts by their respective bioherbicide: sicklepod by *Alternaria cassiae*, northern jointvetch *(Aeschynomene virginica)* by *Colletotrichum coccoides*, hemp sesbania *(Sesbania exaltala)* by *Colletotrichum truncatum*, and Florida beggarweed *(Desmodium tortuosum)* by *Fusarium lateritium* (Hoagland 1996). Synergy of bacterial plant pathogens with sulfosate and glufosinate was demonstrated in greenhouse and field trials. Bacterial strains that caused no symptoms when applied alone dramatically increased the activity of sublethal rates of the herbicides when applied together. They named this approach the "X-tend" bioherbicide system (Zidack and Quimby 1998). Collego® mixed with chemicals used in rice produc-tion (propanil, molinate) and the fungicide benomyl resulted in loss of bioherbicide efficacy. Other herbicides, such as acifluorfen and bentazon, and some insecticides can be applied with Collego in a single tank mixture. Although these tank mixtures broaden the spectrum of insects and weeds control-led, no synergistic interactions were reported (Klerk et al. 1985; Simth 1986). Sequential application of herbicide and pathogen resulted in no inhibition of pathogen growth or activity. This point out that timing is a major factor in integrating pathogens and other chemicals in attempts to find synergy (Hoagland 1996). The precise mechanisms through which mycoherbicide and chemical produce additive or synergistic effects are largely unknown. The pathogen my affect the uptake or transport of the chemical but a common explanation, where the chemical has plant growth-regulatory activity, is that the chemicals inhibits the capacity of the plant to resist infection or grow away from the effects of infection. This mechanism is proposed by Wymore and Watson (1987) to explain the interaction between thidiazuron and *Colletotrichum coccodes* on velvetleaf *(Abutilon theophrasti)*.

Combining living pathogens with herbicides to provide additive or synergistic effects for weed control is a complex undertaking. This complexity arises from the fact that two living and dynamic

Systems (weed and microbes) are involved and each acts or reacts physically and biochemically, not only to environmental conditions, but also to each other and to the potentially synergistic chemical. Several general factors can influence chemical-bioherbicide interactions such as, toxicity of a chemical and its residue to the pathogen, concentrations of chemical and inoculum, timing of chemical and pathogen applications in relation to each other and to weed age, elicitation of weed defense by chemical treatment, etc. (Hoagland 1996).

9 Genetic manipulation of mycoherbicides

Possibilities to improve the effectiveness of mycoherbicides involve the genetic manipulation of the pathogens. There are several potential strategies for developing fungal pathogens for improved efficiency in weed control such as, improvement in the ability to be pathogenic to target plants, improvement in the ability of disseminate, improvement in competitive ability, and improvement in safety. For mycoherbicides it may be possible, for example, to increase pathogenicity by standard selection procedures, by selecting induced mutants or by developing the technologies required for optimising production and stability of the inoculum. Alternatively, improvements might be obtained through genetic means, including sexual and parasexual crossing, somatic hybridisation and the use of recombinant DNA, i. e. by genetic engineering. The latter approach offers unique opportunities but there are public and political pressures against the release of genetically engineered organisms into the environment. Nevertheless, their application to mycoherbicide development will require much more information on the genetic basis of the phenomena to be manipulated. In particular, there is an urgent need to deepen our understanding of the genetic basis of specificity and pathogenicity. At present there

is little information available to the nature of the genes that control either pathogenesis or specificity. However, a wealth of physiological and biochemical data does exist which should allow identification of genes whose manipulation would benefit mycoherbicide development (Greaves et al. 1989; Sands et al. 1990; Sands and Miller 1993; Simms 1993; Bailey 2004).

A highly efficient and reproducible procedure for the transformation of the bioherbicide *Colletotrichum gloeosporioides* f. sp. *aeschynomene* by electroporation of germinated conidia is reported. Optimization of the transformation protocol was facilitated by the use of the green fluorescent protein that helped in the identification of stable transformants and in a fast assessment of transgene expression levels, colony homogeneity and genetic stability.

The method described not only opens up opportunities for the genetic manipulation of *C. gloeosporioides* f. sp. *aeschynomene*, but also provides a framework for the development and optimization of transformation in other fungi (Robinson and Sharon; 1999).

Strategies of coupling virulence genes with failsafe mechanisms to prevent spread (due to broadened host range) and to mitigate transgene introgression into crop pathogens could possibly open a new future to the biological control of major weeds in row crops. For example, virulence was increased by ninefold and the requirement for a long dew period was decreased by introducing the gene Nep1 encoding a phytotoxic protein to an *Abutilon theophrasti*-specific, weakly mycoherbicidal strain of *Colletotrichum coccodes.* Similar results were achieved when Nep1 was transformed into a *Fusarium arthrosporioides* attacking *Orobanche* spp. (Gressel and Amsellem; 2003).

10 Phytotoxins

Microbial herbicides have the obvious attraction that many of them produce phytotoxins. It has become increasingly evident that phytotoxins are important disease determinants. Manipulation of the amount and type of phytotoxins synthesised by biocontrol agents has been a strategy for improving the performance of such products (Cutler 1988; Froud-Williams 1991; Dayan et al. 2000). In at least one case, there has been a question of whether at least some of the efficacy of the microbial herbicide product was due to the presence of the phytotoxin in the formulation. Why not simply use the phytotoxin as one would a herbicide?, that is, biocontrol without biocontrol organisms (Duke et al. 2000). Microbial products offer, perhaps, the best readily accessible source of novel compounds with biological activity towards weeds. Tremendous effort has been expended in chemically characterizing thousands of these compounds, yet comparatively little effort has been made to determine their herbicidal potential. Most of the information available on the phytotoxicity of microbial products is not useful in evaluating their potential as herbicides. In only a few cases in which such compounds have been found to be phytotoxic has a mechanism of action been determined. In the few cases in which a molecular target site has been established, it has generally been one that has not yet been exploited by the herbicide industry (Duke et al. 1996).

The most important phytotoxins examples are bialaphos produced by the soil microbes *Streptomyces viridochromogenens* and *S. hygroscopicus.* Bialaphos is metabolized by peptidases *in planta* to yield the very potent non-selective phytotoxin phosphinothricin which has been chemically synthesized and it is marketed as the herbicide glufosinate. Glufosinate is the only commercial herbicide that targets glutamine synthetase (GS). This enzyme is also inhibited by several natural products of microorganisms, but none of them come close to glufosinate as a viable herbicide (Hoagland et al. 1996; Duke et al. 2000). Phytotoxins also vary in host specificity, ranging from host specificity to having no specificity whatever (Poole and Chrystal 1985; Froud-Williams 1991; Strobel et al. 1991).

Non-host specific toxins are of considerably more interest because they often have the potential for killing a range of weeds without phytotoxicity to crops (Duke et al. 1991). An example of such phytotoxins is tentoxin (a cyclic tetrapep-tide) which is produced by several *Alternaria* species and causes severe chlorosis in many of the problem species associated with soybeans and maize without affecting either crop (Duke and Lydon 1987).

Only a small proportion of potentially useful microbial metabolites have been described herein, but examination of the structures leads to at least four conclusions. First, fermentation products have diverse structures and possess unique biocontrol properties. Second, specific classes of compounds contain congeners that have dissimilar biological activity. Third, several synthetic changes may be made to alter the biological properties of natural products without, apparently, totally destroying the biogradable properties. And fourth, biologically active microbial products offer unique and novel templates on which to base further synthetic work for the pesticide industry. Finally, there remain several significant structures for the further development and there are, in the microbial world, many that are yet to be discovered. It is certain that biodegradable, microbially derived pesticides will

be on the market within the next decade (Cutler 1988; Duke et al. 1996; Duke et al. 2000).

11 Biological weed controls as component of integrated weed management

1.4. Control of the weeds:

Conventional methods of weed control have failed due to one or other resons and therefore, search of an effective, cheaper and ecofriendly way of weed management is a serious agenda in front of scientists every times.

Bioherbicides registered until 2014

There is a long history of research on microbial control agents, it is not always appreciated that obtaining an active isolate is only the beginning of a series of activities necessary for implementing the use of a new mycoherbicide (O'Connell et al., 1996). There are important issues to consider including: mass production, delivery systems and 'laboratory to field' studies, strategies for use, registration and commercialisation (Bateman, 2001).

The level of research reports in bioherbicides research has increased tremendously since the early eighties of the last century. Both the number of weeds targeted for control and candidate pathogens studied has increased. Practical registered or unregistered uses of bioherbicides have also increased worldwide. Likewise, the numbers of U.S. patents issued for bioherbicidal use of fungi and their technology have been increased, perhaps foretelling an increased reliance on bioherbicides in the future (El-Sayed, 2005). Table 4 illustrated registered bioherbicides and their current worldwide status

Biological control through fungal pathogen have proved to a very effective and ecofriendly method for many weeds. It has reached to a point be where several fungal pathogens have been patented and commercialized as mycoherbicides.Mycoherbicide is a component of a broad term 'bioherbicide'. According to Watson (1989) bioherbicide are the living entities used deliberately to suppress the growth or reduced the population of a weed species. The concept of mycoherbicide was first introduced by Daniel *et al.* (1970) who demonstrated 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. Use of the pathogen in a product form and an application technique similar to the chemical tactic are the salient features distinguishing the mycoherbicides from classical agents. This avoids the extended period, host density dependency and environmental control over inoculum build up and spread that suppress disease in a natural or induced epidemic. Fungal strains comercialised patended as mycoherbicides are listed in (**Table- 1)**

Table 1: Fungal Strains Commercialised Patented as Mycoherbicides

Pathogen	**Target Weed**	**Patent No.**	**Date**
Albugo tragopognis	*Ambrosia artemisiifolia L.*	SU343671	1970
Alternaria alternata	*Xanthium spp.*	JP2278978	1987
A.cassiae	*Cassia accidentalis L.* *C.obtusifolia L.* *Croalaria spectablis Roth.*	USA4390360 (CASST)	1983
A.crassa	*Datura stramonium L.*	Usa7092100	1987
A.euphorbicola	*Euphorbia spp.*	US4755208 US4871386	1988
Alternaria zinniae	*Carduus tenuiflorus*	US4636386	1987
Amphobotrys ricini	*Caperonia palustrsi*	US4909826	1990

Arauijiio	*Morrenia odorata*	US4162912	1979
Ascochyta hyalospora	*Chenopodium spp.*	EP296057	1988
Bipolaris sorghicola	*Sorghicola*	US46067621	1986
Cercospora rodmanii	*Eichornia crassipes*	US4097621	1978
Colletotrichium coccodes	*Abutilon theopharsti*	CA1224055 (velgo)	1987
Colletotrichium coccodes	*Solanium ptycanthum L.*	US4715881	1987
C.gloeosporides f.sp.aeschynomene	*Aeschynomene virginica*	US3849104 (collego)	1974
C.gloeosporides f.sp.malvae	*Malva pusilla Sm.*	EP218386 (Biomal)	1987
C.malvarum	*Sida spinosa L.*	US3999973	1976
C.orbiculare	*Xanthium spinosum L.*	AU8818454 (Burr anthracnose)	1989
C.truncatum	*Desmodium tortuosum* *Serbania exaltata*	US4643756 US(NTIS) 7338680	1987 1989
Colletotrichum spp.	*Cyperus rotundus L.*	WO90/06056	1990
Drechslera spp.	*Echinochloa crus-galli*	EP374499	1990
Fusarium lateritium	*Abutilon theopharsti* *Sida spinosa L.* *Anoda cristata Schlecht*	US4419120	1983
F.orobanches	*Orobanche spp.*	SU387689	1973
F.oxysporium	*Echinochloa spp.*	UPO2013367	1990
F.roseum	*Hydrilla verticillata*	US4263036	1981
Fusarium tricinctum	*Cuscuta spp.*	US4915726	1990

Fusarium sp.	*Arrowroot*	JP53099321	1978
Hyphomycetes sp.	*Eleocharais kuroganwi L.*	JPO123850	1989
Phomopsis cirsii	*compositae*	EP136850	1985
Psuedomonas spp.	*Bromus tectoreim L.*	WO89/12691	1989
Puccinia canalculata	*Cyperus esculentus L.*	US4731104	1988
Septoria cirsii	*Compositae*	EP136850	1985
Phtophthora palmivora	*Morrenia odorata*	De Vine	1981
C.glosporides f.sp.cuscutae	*Cuscuta chinensis* *C.australis*	Lubao II	2003
Puccinia canaliculata	*Cyperus esculents*	US4731104	1988
Nectria dittissima	*Alnus rubra*	PFC-Mycocharge	1995
N.dittissima	*Alnus rubra*	PFC-Alderkill	1989
Acremonium diospyri	*Diospyros virginiana*	Distributed by Nobal foundation Oklahoma	1989
Chondrostreum purpureum	*Prunus serotina*	Patented	1999
Phomopsis amaranthuicola	*Amranthus sp.*	Patented	USA, 1960
Colletotrichum gloeosporioides f. sp. cuscutae	odder (*Cuscata* spp.) in soybeans	Probably still available Lubao,	China, 1963
Acremonium diospyri	Persimmon (*Diospyros virginiana*) trees in rangeland	Status unknown	USA, 1960
Alternaria zinnia	*Xanthium spinosum*	US4636386	1987
Chondrostereum purpureum	Deciduous tree pecies in rights of way & forests	Myco-Tech™ paste,	Canada, 2004
Alternaria destruens	Dodder species: in agriculture, dry bogs & ornamental	Smolder,	USA, 2005

	nurseries		
Sclerotinia minor	Dandelion (*Taraxacum officinale*) in lawns/turf	Sarritor,	Canada, 2007

Source: Hasija *et al*. (1994) Pandey *et al*. (2010) Wise, R.M., *et al*. (2007). Senthil kumar, N. (2007).

Integrated weed management is a viable component of IPM which combines use of multiple pest resistant high yielding well adapted crop cultivators that also resist weed competition and precise placement and timing of fertilizers to give the crop a competitive advantage. Successful control of joint vetch and winged primarose was achieved by applying a tank mixture of *C. gleosporoides f. sp. aeschynomene* and *C. gleosporoides sp. jussiae*. Application of the above mentioned fungal formulation along with chemical herbicides has controlled a broad spectrum weed. However, many herbicides are known to increase the pathogenic potential of fungi (Quimby and Walker, 1982). The concepts and literature of reaction between herbicides microorganisms and plant disease have been extensively reviewed in many publications . Attempts have been made to exploit the synergistic interaction of some routinely used chemical herbicides plant growth regulators or surfactant and mycoherbicidal agent such as *Alternaria cassiae, Cercospora palmirosa, Colletotrichum coccoides, C. gleosporioides f.sp. Aeschynomene C. gleosporioides sp. parthenii, C. dematium, Phytopthora palmivora, Fusaruim oxysporum, F. solani and Curvularia lunata* etc. (Hoagland, 1990; Gayathri, 1998).

Biological, technological and economical putrefactive of mycoherbicides have exhaustingly been reviewed in many publications (Templeton *et al.*, 1981, 1979; Hasija *et al.*, 1994; Pandey *et al.*, 2001 Charudattan, 1982, 2001; Charudattan & Dinoor, 2000). In the past several attempts have been made to control weeds with fungal products or mycoherbicides (Wilson, 1969). Several products are now available in the market and many more are in the pipeline (Cullen & Hasan, 1988). The pioneering technology for mycoherbicide production was developed in the USA over a number of years. Species of the genus *Colletotrichum* and their pathotypes have attracted the attention of scientists because of their aggressiveness and their ease of cultivation. The development of the first product `Collego' based on *Colletotrichum gloeosporioides* (Penz.) Sacc. f. *sp*. a*eschynomene* for the control of northern joinvetch (*Aeschynomene virginica*) in rice and soybean in southern USA has been well documented (Templeton *et al*., 1979; Templeton & Greaves, 1984; TeBeest & Templeton, 1985). *Colletotrichum malvarum* has been used as mycoherbicide for the control of *Sida spinosa* in cotton and soybean (Kirkpatrick *et al*., 1982). A number of other fungi are also being tested as mycoherbicides to control exotic weeds for which the use of chemicals is difficult as well as uneconomical (Table-14). A unique approach to control of yellow nutsedge (*Cyperus esculentus*) has been developed in the USA . The indigenous rust fungus *Puccinia canaliculata* (Schw.) Lagerh is employed or manipulated in an integrated weed management strategy. The fungus attacks the leaves leading to dehydration of the roots and tubers as well as inhibiting flowering making the weed non competitive. *Balansia cyperi* has been reported to cause smut disease on *Cyperus rotundus* (Clay, 1984). Considerable work on mycoherbicides for control of Parthenium weed has been carried out in India. Deshpande (1982) appears to have been the first to explore the possibility of exploiting local pathogens, although no specific potential agents were identified. Rajak *et al*. (1990) undertook a survey around Jabalpur (Madhya Pradesh), collecting diseased specimens of *P. hysterophorus* and isolating suspected pathogens. A total of 25 fungal species were identified, the majority being opportunistic necrotrophs. *Myrothecium roridum* Tode ex. Fr. appeared, from the field survey and subsequent pathogenicity tests, to show most potential for mycoherbicide development (Pandey *et al.* 1990, 1992a). From further pathogenicity screening of the other fungi, it was concluded that most of them had the ability to suppress seed germination of *P. hysterophorus* and cause high seedling mortality, whilst a few could effectively kill mature plants, including: *Colletotrichum gloeosporioides* (Penz.) Sacc; *Fusarium oxysporum* Schlect; *Fusarium monoliforme* Sheld, in addition to *Myrothecium roridum* (Pandey *et al*., 1991). The two soilborne *Fusarium* spp., were the subject of a later study and it was considered that, although their biological potential was high, their safety and specificity remained to be evaluated (Pandey *et al.*, 1992b). This group also reported a new collar rot disease of *P. hysterophorus* due to *Sclerotium rolfsii* Sacc. (Pandey *et al*., 1992c) where it was thought to have considerable potential as a mycoherbicide for control of Parthenium weed. Subsequent host range screening showed that isolates

of *S. rolfsii* were pathogenic to a number of crop plants (cabbage (*Brassica* sp.), beans (*Phaseolus* sp.), castor (*Ricinus* sp.) and *Amaranthus* sp.), as well as to other weeds (Mishra *et al*., 1994). However, the possibility of using S. *rolfsii* as a mycoherbicide to control weeds in non agricultural situations was considered a feasible proposition and studies on mass production of the fungus are in progress (Mishra *et al*., 1995). Severe epidemics of powdery mildew caused by *Oidium parthenii* in and around Coimbatore, Tamil Nadu have also been reported (DBT-GOI, 1996). These reports also suggested that one or two disease-causing organisms isolated from the diseased *P. hysterophorus* plants could be a potential source of mycoherbicides. Aneja *et al*. (1994) recorded a new leaf spot disease on *P. hysterophorus* in Haryana State, caused by *Curvularia lunata* (Walker) Boedjin, whilst Dhawan & Dhawan (1995) isolated a range of fungi (13 species) from the phylloplane of *P. hysterophorus* plants also from this region. The latter authors concluded however, that their low virulence and wide host ranges made them unsuitable as candidates for exploitation as mycoherbicides. Other workers in India are pursuing similar lines of research (Aneja, 1991; Anonymous, 1976) but as far as can be ascertained, no formulated product has reached the stage of field testing. It is against this background that at least four Indian research centres have initiated IPM programmes against this weed. Both chemical herbicides and mycoherbicides based on indigenous pathogens, have been evaluated but results have not been promising due to the economics of chemical control, as well as safety, and lack of suitable exploitable fungi (Aneja *et al.*, 1991; Tripathi *et al.*, 1991; Dhawan *et al.*, 1993): "Of the three methods of control, i.e. manual, chemical and biological, emphasis should be made to control it by biological means, either using classical or bioherbicidal tactics because biological control is considered to be the cheapest and most effective method with minimum impacts" (Aneja, 1991). Between 1995 and 96 these centres approached IIBC independently, for guidance in assessing the potential of exotic fungal pathogens as biological agents and for assistance with their supply.

The potential of soil borne fungi to incite diseases in crop plants are well known. Several epiphytotics in plant populations have been reported in the past. These potential have also exploited in weed management. Fungi that are virulent, host specific and genetically stable, but constrained naturally by low inoculum production and poor dissemination are probably the best candidate for development of mycoherbicide (Templeton *et al*., 1986). Efforts made by the weed pathologists reached to a point where four major strategies i.e. Classical, Mycoherbicidal, Biorational and Integrated weed management approaches have been clearly defined (Duke, 1986; Pandey *et al*., 1995, 1996ab, 1997, 2002., Kenfield *et al.*, 1988; Hoagland, 1990; Duke *et al*., 1991; Pandey *et al.*, 1995, 1996b, 2002; Abbas *et al.*, 1992; Van Dyke, 1991; Abaas & Duke, 1997). Some of the well-known examples of fungal bioagents are as follows: *Phytophthora*, a well known soil bornee fungus is responsible for several devastating disease in plants. The first registered commercially available microbial herbicide is the soil borne fungus, *P. palmivora* marketed as DeVine applied to soil around citrus trees for the control of Stranglervine (*Morrenia odorata* Lindl) in Florida. Market product contained wet formulation of chlamydospores (6.7 x 10^5/ml) with a shelf life of six weeks in refrigerated storage. It is an exceptionally effective soil-bornee weed pathogen, and a single application of De Vine™ often gives 95-100% control of the weed for six years or more because the inoculum becomes established in the vine root debris (Kenndy, 1986; Connick *et al.*, 1990). According to Kennedy (1986) improvement in formulation for longer shelf life at ambient temperatures would have been necessary. Another fungus *P. cyperi-rotundati* has been reported to kill purple nutsedge (*Cyperus rotundus*) in India (Seethalakshmi, 1953). *Cephalosporium diospyri* Crandell incite wilt in *Diospyros virginiana* L. (Persimmon) and responsible for substantial control of weed in Oklahoma (Griffith, 1970). The fungal spores are provided in suspension to co-operating growers by the Nobal Foundation, Ardmore, Oklahoma (Templeton & Greavest, 1984). Another wilt inducing strain of *Cephalosporium* is used in control of Kolomona weed (*Casia surrattensis)* a woody plant (Trujllo & Obrero, 1976). Requirement of hand inoculations is one of the major constraints in commercialization of this agent. *Chondrostereum purpureum*, a wound infecting Basidiomycete when applied even at very small dose killed *Prunus serotina* Ehrh. (Blackcherry) effectively in the forest of Netherlands (De Jong *et al.*, 1990; Wall *et al.*, 1992). Initially it act as saprophyte on wounded hard wood and become pathogenic by invading the cambium in the injured areas thus killing resprouts of perennial weeds (Prasad, 1996). It is marketed as Biocon by Kopport Biological Systems (Berkel en Rodenrijs, Netherlands). *Sclerotium rolfsii* a well known necrotrophic fungus incite severe collar rot diseases in many plants including *Parthenium* (Bilgrami *et al*., 1979, 1981; Rajak *et al*., 1990; Pandey *et al.*, 1992b, 1996). Mishra *et al*. (1994) reported significant level of host specificity in this pathogen which further proved

by Shukla and Pandey (2008). According to Mishra *et al*. (1995) it can be stored for a longer duration at lower temperature and moisture in host debris. The pathogen can infect host plant by production of oxalic acid in advance and produce several sclerotia at advanced stage. Physiochemical requirements of two mycoherbicidal strains of *S.rolfsii* have been standarized by Mishra *et al*. (1996b). The agent significantly kill *Parthenium* seedlings when applied @ 90sclerotia/100ml sterilized distilled water. Actively growing mycelium when used as inoculums provides better control than *Sclerotia*. Shukla and Pandey (2008) reported very significant pathogenic diversity in various isolates of *Sclerotium rolfsii* effective against *Parthenium*. Various aspects of mycoherbicidal potential of *S. rolfsii* against *Parthenium* have been extensively been reviewed in many publications (Pandey *et al.*, 1996a, 1999). Pandey *et al*. (2002). also reported significant level of mycoherbicidal activity in *S. rolfsii* isolate against *Hyptis suaveolens*. Various strains of *Sclerotium* spp. synthesize oxalic acid which have been reported to cause severe phytotoxicity and responsible for pathogenesis (Higgins, 1927; Maxwell & Bateman, 1968; Bateman & Beer, 1965). Significant herbicidal activities in crude culture filtrate of *Sclerotium rolfsii* strain containing oxalic acid against weeds have also reported by many workers . Shukla and Pandey (2008) reported very significant control through different formulation of bioactive molecules of *S.rolfsii*. *Sclerotinia sclerotiorum* appears to be among the most non- specific, omnivorous and successful of soil bornee plant pathogen (Purdy, 1979) incites severe stem rot in *Parthenium* (Ghasolia & Shivpuri, 2002). Similarly *S. homoeocarpa* incite severe blight in *Cyperus rotundus* in Mississippi (Bain, 1964).An isolate of *S.sclerotiorum* has also been evaluated as bioherbicide against Canada thistle (*Circium arvense*), spotted knapweed (*Centauria maculosa*) and Dandellion (*Taxanum officinale*) (Brosten & Sands 1986; Miller *et al.*, 1989 a,b; Riddle *et al*.,1991). Repeated application of heat killed perennial ryegrass with *S. sclerotiorum* reduced 80-85% dandellion populations (Riddle *et al.*, 1991). 20-80% control of Canada thistle has been recorded when wheat seed infected or sclerotia of this pathogen applied in the field (Brosten & Sands, 1986). *Centauria diffusa* also severely infected by this pathogen (Watson *et al.*, 1974). The pathogen has a very wide host range, however, a genetic approach has been utilized to make the agent environmentally safe (Miller *et al.*, 1989 a, b; Te Beest 1993).*S. minor* have shown high herbicidal potential against some weeds . Many *Fusarium* spp. have been tried for effective control of several weeds. *Fusarium pallidoroseum* is another important soil borne pathogen reported to be a potential mycoherbicidal agent against *Parthenium* (Kauraw & Bhan, 1995; Farkaya *et al*., 1996; Madhukeshwara *et al.*, 2002; Kauraw *et al*., 1997). Pandey and Pandey (2000) reported very high mycoherbicidal potential of *Fusarium*#LC34 against *Lantana camara. F. oxysporum* and *F.moniliforme* incites severe seedling blight and responsible for more than 90% inhibition in seed germination (Pandey *et al.*, 1991). *F. solani* is also reported as potential pathogen and responsible for significant damage to the weed *Parthenium* (Pandey *et al.*, 1992 a). Farkya *et al.* (1996) reported considerable difference in physiochemical condition suitable for *in vitro* growth and sporulation of two strain of *F.oxysporum*. According to Farkya *et al.* (2001), *F. oxysporum* PR#12 and *F. solani* PR#13 showed maximum mycoherbicidal potential against *Parthenium* seedling in sandy soil, 25^0C, 70% moisture and high RH. The agent has shown significant host specificity against *Parthenium* (Farkya *et al.*, 1994). Abbas *et al*. (1991) reported that an indigenus isolate of *F. moniliforme* caused very high damage to Jimson weed and some other weed. *F. aveneceum* showed very high mycoherbicidal potential against spotted Knapweed (Centaurea *maculosa*) in Montana, U.S.A. (Czembor & Strobel, 1997). The agent significantly reduced the seed germination, overall plant weed growth. *F. oxysporum* and *F. latertium* incite severe blight and have shown promising herbicidal activities against yellow nutsedge (*Cyperus esculentus*) in Georgia (Pathak *et al.*, 1987). A strain of *F. oxysporum* f.sp. *orthoceras* (FOO) has shown promising and an effective mycoherbicide candidate for broomrapes (*Orobanche cumana*) in laboratory, green house as well as in field conditions (Bedi, 1994; Thomas *et al.*, 1998; Bedi & Donchev, 1991). Indigenous isolates of *F. oxysporum* and *F. solani* reduced the number and weight of emerging *O. ramose* shoots and tubercells by 60% and 70%. Similarly *F. camptoceras* and *F. chlamydosporum* responsible for more than 50% control of this weed (Boari & Vurro, 2004). Types of inocula and formulation greatly influenced the short life and efficacy of the bioherbicidal agent.. Amsellem *et al.* (1999) successfully controlled *Orobranche aegyptice* by a formulation containing conidia and mycelia of *F.oxysporum* Foxy with absorbent starch corn oil, sugar and silica. They reported that mycelia based formulation is better storable than conidia. Granular formulation of Pesta containing propagules of *F.oxyporum* Foxy 2 and Foo have also reported to provide significant control of *Striga hermonthica* and

O. cumana (Kroschel *et al.,* 2000; Muller-Stover, 2001;Shabana *et al.,*2003;Elzein, 2003). Upto 100% viability of fungal propagules for at least one year has been reported by these workers. Application of 0.5g granules/kg of soil approximately 300g pesta is required for effective control of the infested *O.cumana* and *S. hermonthica*. Alginate rice *F. oxysporum* prills enhanced the pathogens population in soil incite disease in coca. Connick *et al.* (1998) reported one year shelf life of *F. oxyporum* EN4-S stored with pesta granules at 35^0C ($0.12a_w$) and 2 years at 25^0C (0.12 & 0.33 a_w). Formulation of various strains of *F. oxysporum* has extensively reviewed in many publications (Berger *et al.,* 1996; Elzein, 2003; Elzein & Kroschel, 2003; Elzein *et al*., 2000, 2003). Pesta being free flowing granules has several advantages as it is non toxic, relatively cost effective, easy to mass produce, convenient to store, simple to use, can be easily applied using agricultural machinery. *F. culmorum* has been reported as one of the most lethal to hydrilla and consider as safe agent to control this weed (Charudattan *et al.,* 1980). *F. avenaceum* #1003 responsible for 100% inhibition in seed germination of spotted knapweed (*Centaurea maculosa*) in Montana (Czembor & Strobel, 1997).*F.solani* f.sp.*cucurbitae* has effectively controlled *Cucurbita texana* (Texas gourd) in artificial condition and in field tests (Boyette *et al*.,1984; Weidemann & Templeton, 1988ab). Yu and Templeton (1983) reported that tank mixture or sequential applications of this agent and trifluraolin caused higher and rapid seedling mortality in the target weed. .*F. lateritium* formulated with kaolin (without nutrient) and *F. solani* f.sp. *cucurbitae* with Kaolin and CMC as nutrient provide significant control to *Abutillon theosphrasti* and *C. texan* respectively (Walker & Connick, 1983; Boyette & Walker, 1986; Templeton, 1988ab; Widemann,1988). Quimby Jr. (1985) developed a disperable spray formulation of *F. lateritium* by mixing macroconidia with hydrated silica powder, peptone and starch. Boyette and Walker (1986) recorded significant control of velvetleaf and prickly sida by *F. lateritium* alginate granules. It consider an excellent formulation for applying bioherbicide to soil where effective dissemination of conidia is less of a problem (Connick *et al.,*1990).Similarly tank mixture of *F. lateritium* and acifluorfen controlled 100% prickly sida (*Sida spinosa*) seedling as compared to 8% and 68% for only pathogen and herbicide respectively (Quimby,1985). *Fusarium* spp. are also well known for their production of phytotoxic metabolites such as Fuminosins (Abbas & Boyette, 1992; Abbas *et al.,* 1989), Fusaric acid (Nelson *et al*., 1983; Abbas *et al*., 1989), Moniliformin (Nelson *et al.*, 1983), Enniatin (Burmeister & Plattner, 1987) and Trichothecene (Abbas *et al.,* 1989). Fumonsin B has been reported to caused severe phytotoxicity against many weeds including jimson weed (Abbas *et al.*, 1991, 1995; Duke *et al.*, 1991; Abbas & Boyette, 1992; Tanaka *et al.*, 1993; Vesonder *et al.*, 1992).Pandey *et al.* (2002) reported very high phytotoxicity of cell free culture filterate of *F. solani* FGCC#02 and *F. oxysporum* FGCC#6 against *Lantana camara.* Culture filtrate of *Fusarium pallidoroseum* at 75% and 100% concentration inhibit seed germination of *Phalaris minor* in the laboratory upto 48 and 100% respectively (Kauraw *et al.,* 1997). Manikman *et al.* (1997) reported 70% inhibition in seed germination of Parthenium treated with phytotoxic metabolite of *Fusarium monoliforume*. Several other necrogenic phytotoxin producing fungi have also used to control *Hydrilla verticillata* (Hydrila) at various places (Charudattan & Lin, 1974**).** *Acremonium diospyri***,** *a* wilt inducing fungus, is not commercially available, but is routinely used as mycoherbicide to control persimmon trees *(Diospyros virginiana)* in rangeland in South-Central Oklahoma. Wilson (1965) reported that the fungus has been used since 1960 to control trees upto 10 cm diameter. Hand inoculation of conidial suspension provides 100% control within 3 years (Griffith, 1970). It is provided free to local ranches by the Nobal Foundation near Ardmore, Oklahoma. Loss of virulence is a major problem with this fungus (Templeton & Heiny, 1989). *A. alternatum* recovered from rhizospheric soil caused 80% inhibition in seed germination and 70% seedling mortality in *Parthenium* (Rajak *et al.,* 1990; Pandey *et al.,* 1991). *Rhizoctonia solani* is a destructive, versatile, widespread, noxious soil borne pathogen incites severe diseases in many weeds *viz*; *Parthenium* (Kumar *et al.,* 1979) and *Cyperus rotundus* (Phatak *et al*., 1987). It has high competitive saprophytic ability in soil but required sufficient food base prior to colonisation of host plants (Bateman, 1963; Parmeter & Whitney, 1970). Haygood and Martin (1990) reported significant pathogenic potential of *R. solani* against Centipede grass (*Eremochloa ophiuroides*) and St. Augustine grass (*Stenotaphrum secundatum*). An isolate of *R. solani* has also been reported to cause significant disease in *C. tagetum* . *Aspergillus spp* viz., *A.flavus, A. parasiticus, A. nominis, A. nidulans* and *A. niger* are potential source of Aflatoxins which cause several phytotoxicity in many plants including weeds (Lilly, 1965; Schoental & White, 1965). They are absorbed by the plants and translocated to and distributed within specific cellular compartments (Mclean *et al*., 1994). *Chaetomium globosum,* a very common saprophyte

fungus colonized abundantly on seeds. More than 90% inhibitions in seed germination of *Parthenium* have been reported by (Rajak *et al.,* 1990; & Pandey *et al*., 1991). an abundant colonizer of plant residues, reduced weed population at pre-emergence stage. Chandramohan *et al.* (2002) reported significant control of many weeds by combined application of *Drechslera gigantea, Exerohilum longirostratum* and *E. rostratum. Myrothecium spp.* including *M.verrucaria and M. roridum* are common inhabitant of soil and facultative pathogens of various plants including weeds.Significat herbicidal potential of *M. verrucaria* has been recorded against *Cassia obtusifolia* (Boyette *et al.,* 1991; Walker & Tilley, 1997), *Puereria labota* (Abbas *et al*., 2001), *Euphorbia esula* (Yang *et al.,* 1991) and *Carduus ocanthoides* (Yang, 1994).Anderson *et.al.* (2004) reported high herbicidal activity against metabolites produced by the fungus against *C. obtusifolia. Myrothecium roridum* is a unique agent recovered by Rajak *et al.* (1990) reported to kill *Parthenium* seedlings at both pre and post emergence. It inhibits more than 90% germination of seed and responsible for 100% seedling mortality in this weed (Pandey *et al*., 1990, 1991). Pandey *et al*. (1992) standardized the inoculum doses for considerable level of control of the weed. Many other isolates of *Myrothecium* spp viz., *M. roridium* # FO252 and *M. verrucaria* have also shown very high mycoherbicidal potential against several weeds (Abbas *et al.,* 2002; Boyette *et al.,* 1999; Hoagland *et al*., 2007, Lee *et al*., 2008). *Paecilomyces varioti* a soil borne fungus has recently been reported as potential mycoherbicidal against Horse purslane or Saranai (*Trianthema portrlacastum*) responsible for more than 80 % seedling mortality in the weed (Babu *et al.,* 2003). Another strain of this pathogen i.e. SANK21086 produces some highly effective herbicidal compounds viz; Cornexitin and Hydroxycornexistin against many weeds (Fields *et al.,* 1996). *Phoma* spp are also known to synthesize several phytotoxic metabolites, however, herbicidal properties have not yet explored properly. Herbicidal compound i.e., 3-nitro-1,2 benzene carboxylic acid (3 nitrophthalic acid) synthesized by *Phoma herbarum* FGCC#75 causes severe damage in Parthenium (Rajak *et al.,* 1990; Vikrant *et al*., 2006). Quereshi *et al.* (2006) also reported very high herbicidal activity in CFCF obtained 21 days old fermented broth of *Phoma* sp. FGCC#54 against Parthenium. Herbicidal potential of various spp. of this pathogen have extensively been reviewed by Kovics *et al*. (2005). *Trichoderma* spp viz; *T. viride* and *Trichoderma (Gliocladium) virens* are well known soil borne non pathogenic fungus, *Trichoderma* (*Gliocladium) virens*, produces a broad spectrum herbicidal compounds i.e. gliovirin, gliotoxin, viridian and a metabolic derivative of viridin, viridol which have successfully used for control of many weeds (Jones & Hancock, 1987, 1990; Howell & Stiponvic, 1984). Hutchinson (1999) and Heraux *et al.* (2005) reported very high herbicidal production of viridol on chicken manure. Verma *et al.* (2005) reported significant herbicidal potential of these fungi against Parthenium. Significant reduction in seed germination, root and shoot length of Parthenium and *Phalaris minor* by different applications of *Trichoderma viride* have also been reported by Kauraw *et al.*, 1997..Mode and mechanism of action phytotoxic metabolites have been extensively discussed in many publication (Devine *et al.,* 1993; Duke, 1990; Duke *et al.,* 1991; Cutler, 1991; Saxena & Pandey, 2001; Dayan *et al.,* 1999). *Ulocladium botrytis* a very common fungus incite severe disease in *Orobanche crenata* and responsible for significant control of this weed. There are numerous reports of endophytic fungal parasites of annual and perennial wild plants that prevent their host form flowering or setting seed, and very often these have little visible effect on their host until flowering, particularly species of smut and bunts viz., *Ustilago, Sporisorium* and *Telletia*. In many cases the developing inflorescence is destroyed and a mass and smut spores is produce instead. In others sporulation is confined only to the ovaries, ovules and stamens. Many other fungi viz; *Epichole* sp. belongs to Claviceptaceae also cause sterilization of host and completely suppress the flowering. The key feature of these sterilizing fungi is that infection is systematic, such that the host is prevented form producing seed but instead produces spores of the pathogen. Some of such endophyte has been successfully applied for weed control. *Sporisorium ophiuri* and *Spnaculotheca holci* have been successfully controlled *Rottboellia cochincinesis* and *Sorghum halapense* respectively (Massion & Lindow, 1986).Biology and feasibility of these as bioherbicides have been exhaustively reviewed in many publications including Smith & Halt (1997). Several soil inhabiting micro-organisms have been successfully exploited for microbial transformation of xenobiotic into potential herbicidal metabolites. In addition to providing an alternative to chemical synthesis, the microbial synthesis approach can be used to predict metabolite environment fate studies.

It is evident from the above discussions that despite of several weed problems in a very important crop and excellent potential of fungal pathogen, no serious effects have so for made to discover and exploit fungal pathogens in the management of weeds in this crop.

Thus, the present investigations was undertaken the fungal dicrib to discovered and evaluate there mycoherbicideal potential against prominent weeds of soybean.Integrated pest management for crops is a concept that combines pest control principles, practices, materials and strategies to maintain plant health by minimizing damage from pests. Components of integrated pest management systems vary according to the presence of different modifying factors. Strategies include minimum use of chemical herbicides to maintain pests below economic thresholds, use of biological control agents for specific pests, use of resistant crops cultivars, modification of culture practices to prevent or reduce pest infestation, and the use of any input to prevent the deleterious impact of pests on crops (Shaw 1982; Kendrick 1988). Integrated weed management is a viable component of integrated pest management. The weed management system combines use of multiple-pest-resistant, high yield, well-adapted crop cultivars that also resist weed competition, with precise placement and timing of fertilizers to give the crop a competitive advantage (Smith 1991).Integration of biological control strategies with chemical, culture, and mechanical control practices is essential to a judicious use of biological control in weed and pest management programmes. Because biological strategies control a comparatively narrow spectrum of weed species, chemical herbicides are generally required to control the complex of weed species. Also, few biological control practices are available compared with the many chemical herbicides available for weed control. Therefore, biological control strategies must be integrated with chemical herbicide for effective management of weeds (McWhorter 1984; Charudattan and Deloach 1988; Mueller-Schaerer et al. 2000).Research and development of registered and experimental microbial herbicides indicate that myco-herbicide can be integrated successfully with chemical pesticides into an effective pest management programme. For example, Collego has been integrated successfully with chemical pesticides for control of northern jointvetch as well as other weed species, diseases and insects. As new, improved chemical pesticides are developed for the control of weeds, pathogens, and insects, continued research will be required to determine the effect they have on microbial herbicides or other biological control strategies, and how they can best be integrated into pest management programmes (Hasan and Ayres 1990; Smith 1991; Hoagland 1996; Mueller-Schaerer et al. 2000).

Development of new pathogen strains resistant to improved pesticides offers the opportunity of reducing the adverse impact pesticides have on microbial herbicides. Also, research is required to develop genetically altered pathogen strains that have increased pathogenicity on target weeds, and are compatible with the chemical pesticides used in pest management programmes (Greaves et al. 1989; Smith 1991; Womack and Burge 1993).

Integration of biological control strategies as viable components of weed management programmes will be a challenge to researchers and organizations concerned with pest management sciences. Costs, benefits, and risks of all components of integrated weed and pest management programmess must be examined carefully. Biological control strategies offer opportunities for development of improved weed control practices that will be compatible with all components of integrated pest management systems.

12 Conclusions and outlook

Numerous microbial candidates exist, and preliminary research into biological characterizations has been conducted on these candidates for several decades. The literature is replete with reviews on the subject. Despite all of this research and expense poured into development of microbial biological control agents, very few have been successful and fewer still have persisted in the marketplace. Many candidates have failed, and often for one of multiple common reasons; production problems, lack of stabilization of high titers following fermentation, lack of adequate shelf life of formulations under warehouse temperatures, lack of an economic viable delivery system, or loss of virulence of the product before reaching the target. Therefore, there is a critical need to better understanding of the mode of action of mycoherbicides involved in host-pathogen interactions which consequently leads to enhance the virulence of pathogen and/or suppress the host plant's defence. In fact, there is a substantial difference in studying the mode of action between chemical herbicides and mycoherbicides. In case of chemical herbicides, the active ingredient is a chemical substance, whereas in mycoherbicides, the active ingredients are fungal spores or mycelia. Consequently, the environmental conditions play a basic role in guiding the mode of action of mycoherbicides.

In addition, mycoherbicides require several complex and often specific interactions between the fungus and the target weed. This complexity of interactions is one explanation for the unpredictability and inconsistency often associated with mycoherbicides.

Formulation of mycoherbicides means the blending of active ingredients, such as fungal spores; with inter carriers, such as diluents and surfactants, in order to alter the physical characteristics to a more desirable

form. This may include diluting to a common potency, enhancing stability and biological activity, improving mixing and sprayability, incorporation into granular matrices and the possibility of integrating the mycoherbicide into a pest management system. From my own point of view, research on microbial formulation should be intensified in the forthcoming phase in order to transfer microbial herbicides from the research phase to the implementation phase.

So far, genetic engineering research with mycoherbicide is limited. The virulence genes that enable the pathogens to attack or kill are not well understood. Characterization of these pathogenicity and host-range genes will enhance our understanding of the interaction between a mycoherbicide and a target weed. It will be also expand our ability to generate more effective pathogens. Other genetic traits that may enhance virulence include the use of specific promoters linked to enzyme production and gene products that disengage host responses. The gene encoding production of the natural herbicide bialaphos has been cloned from *Streptomyces hygroscopicus* and transferred into the plant pathogen *Xanthomonas campestris* pv. *campestris.* This herbicide inhibits the enzyme glutamine synthetase and disturbs normal amino acid metabolism. No doubt that using genetic manipulation in mycoherbicides stems from the willingness to find out solutions for the environmental obstacles that encounter a mycoherbicide during field application.

Toxins could represent important tools for improving, directly or indirectly, the efficacy of myco-herbicides. The availability of new methods of purification of toxin and their quantification, structure elucidation, fermentation processing, synthetic production, formulation, knowledge of biosynthetic pathways and molecular tools for their transformation could give further support to the use of these natural metabolites as "helpers" of biological control strategies. The knowledge of toxin structure can permit the preparation of appropriate derivates and/or analogues that are essential to studies of structure-activity relationships, to the understanding of the mechanism of action, to the determination of the active sites of the toxins, and eventually to the production of related toxins having different biological properties. Many studies have shown that changing the active sites of microbial metabolites changes their biological activity.

Much work remains to be done in the use of fungi or microbial toxins for weed control. It is likely, with further refinement of techniques and closer cooperation among plant pathologists, weed scien-tists, formulation chemists and agricultural engineers, that this field will provide fertile sources of alternative weed control methods.

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