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Review

A Brief Review of Innovation with Mycoherbicide used for Biological Control of Lantana Weed

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Abstract: Innovation starts with objectives. We normally find only what we are looking for. Unexpected directions may occur in rare cases. It is however very complicated to rely on unknown events from innovation point of view. Innovators develop their research strategies based on agronomic requirements or demands of the consumer of agricultural products, based on economic expectations, on regulatory restrictions, on research institute strength and on research institute portfolio requirements.

Chemical Herbicides have been indispensable tool for farmers to in developed countries for more than 50 years. High labour costs, energy prices, erosion as an environmental problem and competition resulted in the reduction of mechanical weed measures and resulted in a leading role of chemical herbicides as agrochemicals. Due to the recent trends in environmental awareness concerning the side effects of herbicides, public demands for development of safer, more environmental friendly approaches for weed control. The development of weeds resistant by the application of herbicides demands new alternate to cope with the problem since economic losses generated by weeds can be higher than those caused by other pests. Innovators are continuously searching for identification and characterization of most effective, economical and environmentally safer synthetic herbicides by screening large number of synthetic organic molecules, synthesizing analogs of patent herbicides, designing new herbicide molecules based on target site approach and screening of natural products for friendly trends in weed management force scientists to reach for innovative sources and tools.

Fungi are well recognized for their ability to produce diverse biologically active metabolites including herbicides. The herbicidal properties of fungi can be exploited successfully as atool for the management of weeds. The Large number of secondary metabolites produced by fungi provides ecofriendly, diverse and challenging chemical structures. The biological control of weeds by mycoherbicides (fungal weed pathogens and/their metabolites) have received considerable consideration. Mycoherbicides offer an innovative approach to the management of Lantana weeds using formulated fungal phytopathogen or their natural metabolite extracts would serve as an important component in integrated management strategy. In this research paper, we present the work for the management of *Lantana camara*noxious weeds of India & with isolated indigenous fungal pathogens and by their metabolites.

Keywords: Invasive Alien Noxious Weeds, Lantana, Biocontrol, Indigenousfungi, Mycoherbicide, Marasmins

Introduction:

Invasive alien plants (IAPs) are considered major drivers of biodiversity loss across the globe, and thus efforts have been put in place to mitigate their adverse impacts on ecosystems and human livelihood (van Wilgen *et. al.*, 2018). These efforts address the reduction and/or prevention of new and emerging weeds while controlling existing ones (van Wilgen *et. al.* 2018). In the face of an increasing threat to biodiversity by biological invasions, biological control (biocontrol) is advocated as an essential management tool for selected priority IAPs (Zimmermann *et. al.*, 2004), such as the woody shrub Lantana camara L. (sensu lato) (Verbenaceae), a native to the south and central regions of the Americas (Dey *et. al.* 2003).

Lantana camara, commonly referred to as lantana, is naturalized in over 60 countries in the tropical, sub-tropical and temperate regions of the world, where it is a menace to ecosystems and biodiversity (Day *et. al*. 2003; Urban *et. al*. 2011). Lantana, initially moved around the world by humans for ornamental purposes, and then spread by frugivorous birds and rivers to natural ecosystems (Urban *et. al.* 2011), was declared a noxious weed of the world. Although mechanical and chemical control measures often are used to manage lantana infestations, these options are costly and often inefficient, especially in vast and inaccessible areas (Day *et. al.*, 2003). Biocontrol of lantana is largely affected by the incompatibility between agents and the plethora of lantana varieties, and climatic mismatches between an agent's native range and its country of introduction (Day *et. al.*, 2003).

Finally, fungal plant pathogens that occur locally and have been observed to be damaging to IAPsin the field, have been isolated and considered for their potential as mycoherbicides. Their spores or secondary metabolites can developed as a product to apply to the relevant weed species, with the advantage that they are usually host specific and thus do not damage surrounding vegetation, unlike chemical herbicides. Another advantage is that because they are locally isolated organisms, it is not necessary to work in quarantine or to apply for permission to release them as biocontrol agents in India.

Many research works have shown that invasive plant species have broad distribution throughout the world and can directly or indirectly affect the food security of local residents by destroying natural pasture, displace native trees, crops, and reduce grazing potential of rangelands and set limitations for economic development.

Globally, the extent of damage caused by invasive species has been estimated to be £1.5 trillion per year, close to 5% of global GDP. In developing countries, where agriculture accounts for a higher proportion of GDP, the negative impact of invasive species on food security and economic performance can be even greater which exacerbate poverty (Pimentel et al., 2000). Due to its strong allelopathic properties, aggressiveness and its dense impenetrable thorny thickets, Lantana camara has the potential to interrupt the health and regeneration process of other species by decreasing germination, growth of seedlings and biomass production which in turn increases mortality and decline of plant species, pasture and crops (Sharma and Raghubanshi, 2006). A native of Central and South America (Day et al.,2003), *Lantana camara*, as coined by Carlous Linnaeusin 1753 (Swarbrick et al., 1995), contains approximately 270 species and subspecific taxa of woody shrubs (Bhakta and Ganjewala, 2009).

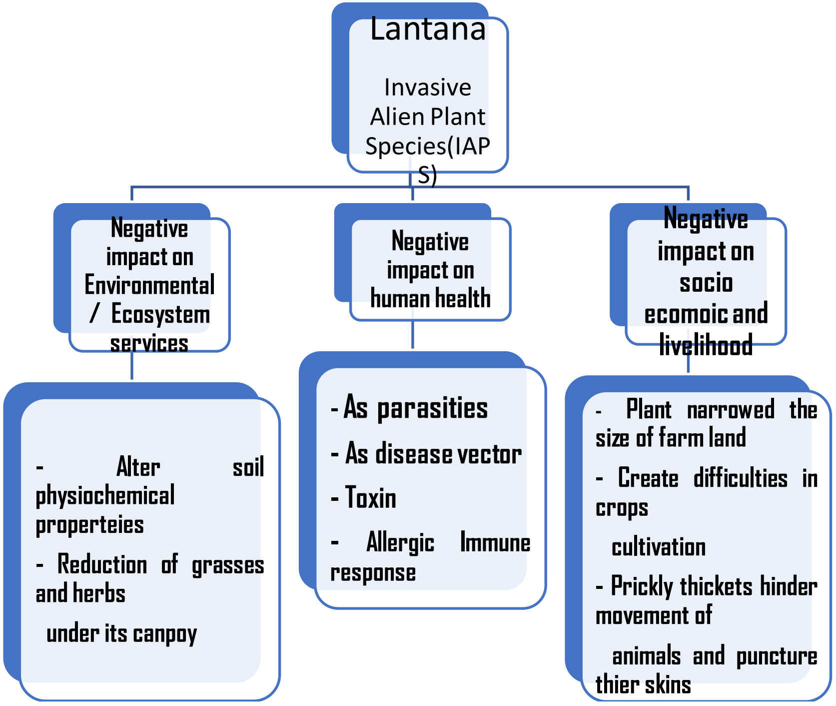

Fig 1. Negative impact of Lantana on Environment, Human health and Socio economic & livelihood

The genus Lantana ranked among the 100 worlds worst invasive alien species (Day et al., 2003). The diverse and broad geographic distributions of the species beyond its native range are the reflection of its wide ecological tolerance, ability to conquer diverse habitats and its success on a variety of soil types (Day et al., 2003). It is now a cosmopolitan exotic invader and has been declared as a noxious weed inmany parts of the world (Goulson and Derwent, 2004)

L. camara (L.), a highly aggressive exotic environmental weed in many countries, has significantadverse effects on biodiversity. It forms dense thickets, suppressingnative vegetation and seedlings through shading, nutrient competition, smothering and allelopathy. The present study has demonstrated that L. camara impact negatively on native vegetation structure and composition. The effects on native vegetation are direct, through smothering and allelopathic means, and indirect through changes in soil properties. However, the result of this study indicates that L. camara has no equal distribution on each land use types and so does it impact equally. The plant was highly abundant and distributed in the grass, agricultural and forest lands of the study area respectively, due to absence of shade effect on the grassland and frequent disturbance in the former land uses (Fig 1). L. camara occupied 62.20% (1379 individuals/ha) of area coverage in the sampled study area (1.44 ha), with high proportion of its seedlings (60.20%). This implies that the greater regeneration In order to meet the objectives of good health and food, weeds must be controlled intelligently. The conventional methods of weed management are operative since long and played a significant role in weed management programme. However, the conventional methods of weed control have various drawbacks. Biocontrol of weed management is application of natural enemies to control the weed growth or suppress weed population. Generally, two basic methods implement for weed control with pathogens. The application of foreign pathogenic organisms generally known as classical approach and where the pathogenic organisms are already present (native) known as bioherbicidal approach (Watson and Wymore 1990; Mueller-schaerer and Frantzen 1996; Mueller-Schaerer and Scheepens 1997). Such pathogens or biological agents are generally "manufactured", formulated, standardized, 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 is mycoherbicide.

Mycoherbicide

Mycoherbicide 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). Mycoherbicide 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; capacity and potential threat of the weed on the environment.

Watson and Wymore 1990). The concept of mycoherbicide 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 approach 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 efficacious under sufficient different environment conditions to allow a feasible application window (Daniel et al. 1973; Templeton et al. 1979). 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 mycoherbicidal technology have increased, perhaps foretelling an increased reliance on mycoherbicides in the future. Currently two mycoherbicides, DeVine® and Collego®, are used commercially in the United States to control, milkweed vine, *Morrenia odorata* in citrus groves of

Florida and northern joint vetch, and *Aeschynomene virginica* in rice and soybean fields of Arkansas and neighboring states respectively (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 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 cassia* 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* (Weidmann1988; Weidmann and Templeton1988); *Lasiodiplodia theobromae* for *Parthenium hysterophorus* (Kumar and Singh 2000); *Fusarium oxysporum* for the narcotic plant coca, *Erythroxylum coca* (Gracia-Garzaand Fravel1998); 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.

Marasmins (Natural Product of fungi)

Fungal metabolites as herbicides have the obvious attraction that many of them produce phytotoxins. It has become increasingly evident that fungal phytotoxin are important disease determinants. Manipulation of the amount and type of phytotoxins synthesized by bio-control agents has been a strategy for improving the performance of such products (Cutler 1988; Froud-Williams 1991; Dayan et al. 2000). Tremendous effort has been expended in chemically characterizing thousands of these compounds, yet comparatively little effort has been made to determine their herbicidal potential. 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). Phytotoxins also vary in host specificity, ranging from high 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). The phytotoxin are biodegradable, microbially derived pesticides will be on the market within the next decade (Cutler 1988; Duke et al. 1996; Duke et al. 2000).

Lantana weed in India and the Potential of indigenous mycoherbicide development

The chemical application have health and environmental problems, in order to reduce chemical application, we have to consider the use of alternatives to chemical herbicides. Under this condition, mycoherbicide and their metabolites are attractive alternative to chemical herbicides. The need to provide alternatives to chemical herbicides is being addressed in a various countries. I have initiated aresearch to investigate the potential for the use of mycoherbicide or their metabolites against some noxious weeds of Madhya Pradesh during my Ph D work. The Lantana weeds are included here because it is not only weed in India but they have presence worldwide.

Lantana camara is a native of tropical America, and was introduced to India as an ornamental to be planted in gardens and hedges. Since then, the species has spread rapidly into both farm and forest lands, and is one of the most widespread, terrestrial invasive species in India today. It is considered as one of the world’s 100 most invasive species, and among the world’s 10 worst weeds. Lantana grows on all types of well-drained soils and in a wide rainfall range(from seasonal dry forests to rainforest) but is also very drought-resistant. It rarely invades undisturbed, closed-canopy forest but rapidly colonizes gaps, edges and disturbed or logged habitats. It produces large numbers of seeds that are dispersed by birds and the seeds germinate rapidly and easily. Lantana is a very efficient competitor against native species under conditions of high light, soil moisture and soil nutrients. It can become the dominant understory species in infested areas, blocking natural succession processes, and reducing biodiversity. It may be threatening the wildlife habitats in forests, and thereby threatening important wildlife populations. Present control methods are limited to physical removal and multiple application chemical herbicides. Plants larger than the fifth leaf stage are difficult to control with any of the commonly used herbicides. Significant herbicidal property in Cell free extract (CFE) obtained from 21 day old fermented broth of *Aspergillus* spp., (*A. nidulans, A. niger, A. terreus, A. fumigatus* and *A. flavus*) against *L. camera* was recorded by employing shoot cut bioassay technique. It was observed that cell free culture filtrate of different species of *Aspergillus* had varied degree of toxicity against *L. camera*. Twenty one days old culture filtrate of *A. nidulans* induced maximum toxicity in the target weed. It was followed by cell free culture filtrate obtained after 14 days of incubation. There was significant reduction in chlorophyll and protein content (Pandey et al., 2005). Saxena et al., (2001) while screening the herbicidal substances secreted by microbes found that the culture filtrate of an indigenous isolate of *Alternaria alternata* SSLC#103 exhibited marked phytotoxic effect against the weed *L camera* 41.62% and 52% change in biomass was recorded after 36 hours post-treatment and at 50% and 100% cell free filtrate concentrations respectively during the *in vitro* whole plant bioassay. Partial purification of the cell free culture filtrate yielded four fractions, of which phytotoxicity resided in the Fraction A and it was a fatty acid. The shoot cut bioassay of this fraction caused more prominent phytotoxic damage when compared to cell free culture filtrate (CFCF). Two species of *Fusarium* viz. *F. oxysporum* and *F. monilifrome* were isolated from the infected leaf of Lantana and evaluated for biocontrol potential.

Both the species caused severe wilting. The pathogens exhibited considerable potential as biocontrol agents (Singh 2007).

Discussion:

Numerous microbial candidates exist, and preliminary research into biological characterizations has been conducted on these candidates for several decades. 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 mycoherbicide involved in host-pathogen interactions which consequently leads to enhance the virulence of pathogen and/or suppress the host plant's defense.Consequently, the environmental conditions play a basic role in guiding the mode of action of mycoherbicide microbial metabolites (marasmins) could represent important tools for improving, directly or indirectly, the efficacy of mycoherbicide. 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 toxins for weed control. It is likely, with further refinement of techniques will provide fertile sources of alternative weed control methods. In addition, biological and cultural control were insignificant in reducing weed populations. The weed can significantly reduce crop yield and quality due to its aggressive growth habit, competitiveness and allelopathic interference. It is a difficult weed to manage, and a wide variety of methods, starting with prevention and containment, are necessary to reduce the incidence and spread of this weed. An integrated approach using cultural, physical, chemical and biological approaches are necessary for the successful management of this weed. Integrated approaches following different methods coupled with proper land management and best management practices can effectively control this weed. Despite the negative impact of this weed on the biodiversity, there is potential in exploring its beneficial properties as a mechanism of management.

Conclusion:

There is no doubt the extraordinary fungal diversity in ecosystem and thus, each pathogen must be considered as unique and must be thoroughly studied laboratory growth chamber or green houses to understand its disease cycle and potential as herbicide. The potential of particular genus as microbial herbicide can be obtained from knowledge about diseases of economic crops incited by other species or forms of the genus. Proper understanding of the disease cycle of a pathogen to be developed as mycoherbicides is very important step in a success of a programme.

The interaction of the life cycles of the fungus and host plant must also be understood. Important facets include the source of primary inoculum, the method of dissemination of infectious propagules, the climatic parameters that favor rapid infection and disease development, the age and physiology of the host that favors or suppress plant infection, variation in genetic resistance of the host or virulence of the pathogen, the method and rapidity of secondary spread and the means of over wintering. Particular emphasis is placed on the climatic parameters, principally temperature and moisture that affect the disease cycle. With the above information together with knowledge of the climate in the geographic region where the weed grows and the growth stage during which the weed must be controlled, a fairly accurate assessment for the mycoherbicides potential of a particular fungus can be made. Unfortunately, many of the published reports that suggest specific fungi as potential mycoherbicides have not researched disease cycle or the weed biology adequately to make a definite judgment of the biological potential of a particular fungus (Templeton et al., 1998). A wealth of knowledge about disease cycles can also obtained with pathogens of economically important crops. However, this knowledge cannot be extrapolated too for because the crop pathogen relationship of disease is usually different than the weed pathogen relationship. Microorganisms specially fungi and actinomycetes are known to produce variety of phytotoxic metabolites with herbicidal properties (Abbas &Duke, 1997; Culter, 1998; Duke, 1986 a,b; Hoagland, 1990, 1999, 2000, 2001; Joseph et al., 2002). Still only few have been screened. Therefore, lot of opportunities exists in their integration with mycoherbicidal agents. Inadequacies discussed earlier may be amenable to correction either by advances in formulation technology for biological or by advanced molecular techniques (Yoder, 1983; Yoder & Turgeon, 1985). Similarly orphaned mycoherbicides can be considered to represent excellent opportunities for a company specializing in a particular group of organisms or a public agency or grower organization interested in providing a service for a specific grower clientele. They may also offers opportunities for biologically active metabolites with weed control potential. Mycoherbicides present suitable opportunities for return on investment from small marketbecause the cost of developing them may be less than that for a chemical herbicide. Production technology is already available in fermentation industries, thus capital investment for production is low. Registration costs could be significantly less than for synthetic herbicides. Time required for research and development of a potential agent through registration and commercial use may be substantially less than for herbicides, and this would represent a significant saving of developmental costs (Templeton et al. 1986). Although, mycoherbicides have proved to be effective, but there is a need for technological improvement with chemical enhancer, by strain improvement or by combining fungi to increase the spectrum of weed control. Many fungal pathogens of weeds may be weed without additional technological improvement. However activity of many other fungal pathogens is supported by low virulence, stringent temperature and moisture requirement, wounding requirement or specific physiological requirement of the host plant. Experience with Collego, Devine, Casst and Bio Mal leaves no doubt that mycoherbicides are effective and practical as weed control agents

(Bannon 1986; Bowers 1986; Bowers 1982; Charudattan et al 1986; Kenney 1986., Ridings 1986., Ridings et al 1976; Smith 1982; Smith 1986; Templeton 1982; Walker and Riley 1982). The chemical industry is known to screen thousands of chemicals for every commercially feasible herbicide. When viewed in this light, mycoherbicides have had a remarkably high rate of return on scientific and monetary input. Experience with agents like *Alternaria cassiae, Cercosporarodamanii, Colletotrichum coccodes* and *C. gloesporioides* f. sp. *malvae* suggest that we are indeed witnessing this second phase of growth in mycoherbicides in which challenges, both scientific and commercial are being posed. The future direction of mycoherbicide is being influenced by current scientific, practical and government decisions (Charudattan, 1984).On the research front following are emerging future outlooks in mycoherbicide research:

- Screening of more mycoherbicide candidates of important weeds. These in turnwill provide a deeper understanding of mycoherbicides.
- Application of mycoherbicides with chemical pesticides. Thiswill be mandated by the fact that each weed – mycoherbicide- pest management systemwill be different and specific recommendations for the use of mycoherbicides will beneeded.
- Application of mycoherbicides with chemical plant growth regulators for improvedweed control through decrease in weed growth and increase in mycoherbicideefficacy. Weeds possessing high raters of vegetative growth and vegetative proliferationtend to be difficult to control with mycoherbicides. The ability to outgrow diseasepressure is a characteristic of these weeds (Charudattan et al 1985; Winder &Dyke,1989). In such cases the integration of mycoherbicides with plant growth regulators, which by themselves may not afford weed control, offer a useful solution (Charudattan,1986).
- Potential Formulation development to improve viability, efficacy and ease of application of mycoherbicides. The need for optimum moisture and specific temperature regimes for infection pose problems in assuring mycoherbicide efficacy. The lack of proper epidemiological conditions for infections and disease development and theadverse effect of solar radiation on fungal propagules can be counted to an extent through formulation technology. Substances that improve moisture retention, reduce drying and UV–irradiation, dilute and evenly disperse the inoculums and provide better host-pathogen contact are being studied (Connick et al 1989).
- Discovery of host specific and non-specific herbicidal metabolites of microbial origin that could be used as virulence and host specificity factors for genetic engineering. It will also solve the problem of optimum moisture, temperature requirement for infection development in mycoherbicide case.
- Improvement of Mass production technology- Current industrial preference favours submerged liquid fermentation to produce mycoherbicides products (Churchill, 1982; Templeton et al,1980). Although successful, cost effective and readily available, this technique is not suitable for fungi that do not sporulate in submerged culture. Solid substrate culturing and airlift fermentation can offer solutions.
- Molecular genetic basis of virulence and host specificity-Genetic improvement of mycoherbicide candidates through bioengineering for increased virulence and increased or decreased host specificity deserves research emphasis. With several mycohererbicide candidates the level off virulence is less than desirable. By incorporating genes for virulence factors

such as host- specific toxins and phytotoxic metabolites or host receptors it should be possible to improve weed control ability of these candidates. On the other hand, several highly virulent and destructive pathogens exist that are suitable as mycoherbicides on account of their broad host range. Mutation- selection, gene cloning, interspecific and intragenic protoplast fusions, electroporation and other methods can be useful for this purpose.

- Increased public and private funding as well as administrative support for research and development of mycoherbicides.
- Education of scientist unfamiliar with mycoherbicides and the user public, which is required for technology transfer- Mycoherbicides, like many other biocontrol agents are sensitive to environmental conditions and need to be handled in strict accordance to the prescribed methods.
- They are usually slower in eliciting the desirable results. The more difficult challenge maybe to convince the agricultural community that crop yield can be improved without killing weeds (Auld & Morin, 1995). The users must therefore, be educated about the use and performance features of Mycoherbicide.

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