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Microbial Herbicides for Weed Management: Prospects, Progress and Constraints

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Abstract: Application of microbial herbicides for the management of agricultural weeds is an eco-friendly approach. A worldwide programme has been growing up to control the invasive weed species for the better crop production and stable ecosystem. Classical bio-control approach is not at all successful over the bio-herbicide approach. Although, a number of microbial herbicide has been developed to till date, only a few of them are available in commercial forms due to several constraints in the formulation, application and commercialization. Biocontrol agents probably fail to be marketed internationally as these are living organisms and are fearful to introduce them from foreign countries. Screening and genetic modification of potent microbial species are highly encouraged for a better commercial mycoherbicide development.

Key words: Bio-control, bioherbicide, classical weed management, formulations, mycoherbicide

INTRODUCTION

Weeds cause serious ecological problems and are capable of altering the process of ecosystem, displacing the native flora and fauna. They may also support populations of non-native animals and microbes and hybridize with native species subsequently altering gene pools (Randall, 1996; Mahanta *et al*., 2007). Out of about 30,000 species widely distributed weeds, 1800 species cause yield loss by about 9.7% of total crop production every year in the world (Li *et al*., 2003). Weed can reduce crop yield by as much as 12%, which results to $32 billions in losses (Anonymous, 1998) as a whole. In 1980s, growers spent over $3 billions annually for chemical weed control, $2.6 billions for cultural, ecological and biological control method (Ross and Lembi, 1983). Non native weeds species are spreading and invading in United State wild life habitat at the rate of 7 lakhs ha/year (Babbitt, 1998), whereas 1 lakh ha/year in Europe, changing the basic structure of wetlands (Thompson *et al*., 1987). Weeds also serve as reservoir for plant pathogens that may cause significant economic loss in crop production. Besides these, weeds also cause environmental impact associated with its management such as non-target injury of living beings, contamination of ground and surface water etc. (Turnera *et al*., 2007). In this context, biological weed management practice seems to be a selective process against targeted weeds without damaging non-target living beings and environment.

Biological control is the deliberated use of living organism to control a pathogen or weed (Tamuli and Boruah, 2000; Hallett, 2005; Chutia *et al*., 2006). During last two decades biological weed control has received considerable attention. These has been results of the intensive use of chemical herbicides coming under scrutiny due to an increasing number of resistant or tolerant weeds (Heap, 1996), effect of non-target organisms, contamination of soil, ground water and food etc. Biological weed control practices have been developed for the sustainable use of biodiversity for economic benefit towards mankind. Insects have been used successfully in bio-control of weeds for many years (Wilson, 1964; Wasphera, 1982). The idea of using plant pathogens for management of weeds was reported before the turn of the century, but it is only in the last three decades that has received increasing interest (Charudattan, 1991; Watson, 1991; TeBeest, 1996).

Inoculative biological control and bioherbicide approach are the two steps in the microbial management of invasive weeds that have been applied successfully (Boyetchko and Rosskopf, 2006). Classical or inoculative biological control is the introduction of exotic organisms as a pathogen to the targeted weed species whereas, in bioherbicide approach microorganisms are multiplied artificially and applied to weeds in a manner similar to chemical herbicides (Mortensen, 1998).

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Table 1: Classical approach of biological weed control practice under major projects

Name of the weed species	Types of infection	Name of the pathogen	Country	Reference
Chondrilla junces L.	Weed rust	*Puccinia chondrillina* Bud and Syd	Australia	Hasan (1972)
Rubus constrictus L.	Black berry rust	*Phragmidium violaceum* (Schultz) Winter	Chile	Hasan (1988)
Acacia saligna (Labill) Wendl	*Acacia* gall rust	*Uromycladium teperianum* (Sacc) Mc. Alp	South Africa	Morris (1996)
Carduus thoermeri Weinm	*Carduus* rust	*Puccinia carduorum* Jacky	North America	Politis *et al.* (1984)
Centaurea diffusa Lam	*Centaurea* rust	*Puccinia jaceae* Otth	Rumania	Watson and Alkhoury (1981)
Ageratina riparia (Regd) K and R	Rust	*Cercosporella* sp.	Hawaii and Jamaica	Trujillo (1985)
Lantana camara L.	Rust	*Septoria passiflorae* Syd	Hawaii	Mortensen (1998)

INOCULATIVE BIOLOGICAL CONTROL

The bio-control approach using a pathogen imported from a foreign location to control a native or neutralized weed with minimal technological manipulations has been termed as classical or inoculative biological method. The overall success of classical biological control projects using imported pathogens has been estimated about 57% (Yandoc *et al*., 2006b) whereas, 30-35% in insect based weed bio-control projects. These success rates are calculated from the number of projects for which success can be verified from published accounts or reliable anecdotes compared to the number of known projects (Charudattan, 2005; Yandoc *et al*., 2006a).

A careful evaluation of efficacy and safety must precede a pathogens introduction, therefore valid protocols based on conceptual frameworks as well as empirical examples exist for selection of safe and effective agents (Watson and Wymore, 1990; Bickie and Morrison, 1993; Berner and Bruckart, 2005; Turnera *et al*., 2007). Precise identification of the race genotypes of the pathogens and confirmation of their virulence towards the target genotypes is important because the host pathogens specificity can be governed by single gene differences or by a small number of genes, particularly at the sub specific level (Yandoc *et al*., 2006a).

Classical bio-control of weeds began in 1960s on *Rumex* sp. in United States (Inman, 1971) and *Rubus* sp. in Chile (Oehrens, 1977). Since then lots of work has been done successfully (Table 1) in this programme (Bruckart and Hasan, 1991; Watson, 1991; Barton, 2005; Charudattan, 2005). Among the most successful is the control of *Acacia salgina*, *Chondrilla juncea*, *Ageratina riparia*, *Carduus thoermeri*, *Cryptostegia grandiflora*, *Baccharis halimifolia*, *Mimisa pigra* etc. by the different plant pathogen (Cullen, 1985; Trujillo *et al*., 1988; Baudoin *et al*., 1993; Bruckart *et al*., 1996; Luster *et al*., 1999; Morris *et al*., 1999). *Uromycladium tepperianum* was introduced as rust fungus for *Acacia saligna*, an invasive weed that threatens the Cap Fynbos Floristic Region of South Africa. This pathogen significantly decreased the tree density by 90-95%. The bio-control project to control *C. juncea* (rust skeleton) by *Puccinia condrillina* has been estimated to yield a cost of benefit ratio 1:100 to 1:200 (Cullen, 1985). Another successful weed bio-control programme has been achieved by the use of foliar smut fungus *Cercosporella* sp. in Hawaiian forest from Jamaica to control *Ageratina riparia* (Trujillo *et al*., 1988). It was estimated that more than 50,000 ha of pastureland have been rehabilitated to their full potential due to the application of this pathogens. No evidence of host resistance or the presence of mutant stains of the pathogens has been encountered (Trujillo, 1985, 2005). *Puccinia jaceae var solstitialis* is the recent introduction to the United States from Bulgaria and Turkey and the host range tests on these pathogens were extensive (Bruckart, 2006). A major project has been undertaken for biological management of *Mimosa invisa*, a non-native invasive species that threaten to the Kajiranga National Park in India. Several other projects have also been undertaken worldwide for the management of invasive species using microbes, insects, phytoextracts etc.

BIO-HERBICIDE APPROACH

Microbial preparation of herbicide is defined as bioherbicides that can control the weed (Li *et al*., 2003). In this approach, indigenous plant pathogens isolated from weeds are cultured to produce the large numbers of infective propagules which are applied at a rate that will cause high levels of infection leading to suppression of the target weed. It is estimated that there are over 200 plant pathogens that have or are under evaluation for their potential as bio-herbicides; these include fungi and bacteria that cause foliar disease, soil born fungal and bacterial pathogens and deleterious rhizobacteria (DRB) (Rosskopf *et al*., 1999; Charudattan, 2001; Boyetchko *et al*., 2002). Sauerborn *et al*. (2007) described the virulence of parasitic weeds, their occurrence and parasitic life-style which make them suitable targets for biocontrol using the bioherbicidal approach-multiplication and periodic release of indigenous microbial agents for sustained control of the target species. A list of registered and commercially produced mycoherbicides are shown in Table 2. These plant pathogens based herbicide are generally evaluated for their virulence, performances under field condition, host range specificity etc.

Table 2: Status of microbial commercial bio-herbicide since 1964

Name of microbes	Name of Bioherbicide	Target weed species	Registration detail	Present Status
Acremonium diospyri	-	Persimmon trees in rangelands	USA, 1960	Unknown
Collectrotrichum gloeosporioides f. sp *cascutae*	Libao	*Cuscuta* sp.	Chaina, 1963	Probably still available
Phytopthora palmivora	DeVine^{TM}	*Morrenia odorata*	USA, 1981	Status unknown, may no longer be marketed
Collectrotrichum gloeosporioides f. sp. *aeschynomene*	Collego	*Aeschynomene virginica*	USA, 1983	No longer been available due to commercial backing
Puccina cnaliculata	Dr. BioSedge	*Cyperus esculantus*	USA, 1987	Registered, but product failed to uneconomic production system and resistance in some weed biotypes
Collectrotrichum gloeosporioides f. sp. *malvae*	BioMal^{TM}	*Malva pusilla*	Canada, 1992	Not commercially available but recently taken on by a new financial backer who is exploring market opportunities
Cylindrobasidium leave	Stumpout^{TM}	*Acacia* sp.	South Africa, 1997	Still available
Chondrostereum purpureum	Biochon^{TM}	*Prunus serotina*	Netherlands, 1997	Available until 2000, production stopped due to low sale
Xanthromonas campestries pv. *poae*	Camperico^{TM}	*Poa annua*	Japan, 1997	Probably commercially available
Collectrotrichum acutatum	Hakatak	*Hakea gummosis* and *H. sericea*	South Africa, 1999	Not registered but will be produced on request
Puccinia thlaspeos	Woad Warrior	*Isastis tinctoria*	USA, 2002	Registered but commercially not available
Chondrostereum purpureum	Chontrol^{TM} Ecoclear^{TM}	Alders and other hardwoods in rights of way and forests	Canada, 2004	Commercially available
Chondrostereum purpureum	Myco-Tech^{TM}	Deciduous tree species in rights of way and forests	Canada, 2004	Commercially available
Alterneria destruens	Smolder	*Dodder* species	USA, 2005	Just registered and company planning to do more field trials in 2007

Out of these hundreds of bio-herbicides a few of them are commercially available. Stumpout (*Cylindrobasidium leave*), Ecoclear^{TM} (*Chondrostereum purpureum*) and MycoTech^{TM} paste are three commercially available bio herbicide (Barton, 2005). Smolder, a bio herbicide from *Alterneria destruens* has been registered recently and company planning to do more field trials and then market it in 2007. The others are unavailable due to the lack of continued commercial backing, high cost of mass production, introduction of newer herbicidal chemistries, resistant biotype (e.g., Dr. BioSedge) or limited markets. One herbicide agent, *Collectrotrichum gloeosporioades* f. sp. *Aeschynomene* has been reregistered (previously Collego) as of March 2006 under the commercial name LockDown for use in the rice in Arkansas, Lousianna and Mississippi (Yandoc *et al*., 2006b). All these herbicide have potential weed control capacity up to 100% in field condition though its efficacy regulated by inoculum's concentration, formulation, spray parameters, target weed plant age, non-target plant species, micro and macro organisms in the phyllosphere or rhizosphere and pesticides applied in the area.

Many species of weeds were reported to acquire resistance against commercially available chemical herbicides. There are about 307 herbicide resistant weeds biotype worldwide, 113 of these biotypes occurs in the United States alone (Heap, 2006). Today it is possible to improve efficacy of plant pathogens by recombinant DNA technology. Charudattan and Dinoor (2000) has modified the host range to improve virulence of *Xanthomonas campestries* pv. *Campestries* (host *Poa annua*) by using gene encoding bialaphos production to control weed as some biotypes have developed resistance to a number of herbicide families. Loretta *et al.* (2006) described that 7 species of *Amaranthus* had become resistance to a number of herbicidal families. But the combined application of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* as a mixture had significantly decreased the weed species in the field (100% mortality).

FORMULATION OF BIO-HERBICIDE

The formulation of bioherbicide is the blending of the active ingredient, the biological propagules with a carrier or solvent and often other adjuvant to produce a form which can be effectively delivered to the target weed (Boyette *et al.*, 1991; Rhodes, 1993). Most of the formulations of the biological control agents are largely based upon techniques developed for formulation of

Table 3: Formulating substrates /medium of potent bioherbicides

Type of formulations		Substrate/medium of the bio-herbicide formulation
Liquid	Water	Distilled water (Connick *et al*., 1990)
	Adjuvant	Bio-surfactants (e.g., surfactin by *Bacillus subtilis*), oxysorbic polyoxyethylene sorbitan monolauruate, oxysorbic polyoxyethylene sorbitan monoleate, polyglycerol etc. (Foy, 1989; Prasad, 1994)
	Inverted emulsions	Water suspended oil, soyabean lecithin, paraffinic oil, paraffinic soyabean oil etc. (Womack *et al*., 1996; Yang and Jong, 1995).
	Oil suspension emulsion	Corn oil, coconut oil, wheat germ oil, cotton seed oil, fish oil, sunflower seed oil etc. (Auld, 1993; Egley and Boyette, 1995)
	Wettable powder	Aluminium- silicate powder. (Boyette *et al*., 1996; Mortensen, 1998)
Solid	Alginate formulation	Sodium alginate, hydrous aluminium silicate etc. (Bashan, 1986)
	Other granular formulation	Corn starch, corn flour etc. (Boyette *et al*., 1984; Zimmermann, 1993)

agrochemical (Cook *et al*., 2005) involving the use of organic solvents, surfactants and drying methods, which can be detrimental to biological propagules (Connick *et al*., 1991). Majority of bioherbicide formulations are concentrated of maintaining agent viability in storage and reducing dew period requirements (Green *et al*., 1998). Liquid and solid formulations of bioherbicides are the two different approaches for infection in above and below ground parts of the weed species.

Liquid formulation: Liquid formulation includes aqueous, oil or polymer based products, oil suspension emulsion, inverted emulsion etc. tend to be used as post emergence sprays to incite leaf and stem diseases on the weed host (Boyette *et al*., 1991; Womack *et al*., 1996). Water is the simplest bio-herbicide delivery system contains the propagules of the agent formulated as spray able suspension in water (Connick *et al*., 1990). Application of adjuvant for bioherbicide formulation assists or modifies the action of a principal active ingredient (Foy, 1989). This encompasses a wide range of compounds (Table 3). A variety of microorganism produces some potent surfactants and can be used as bio surfactant in herbicide formulation (Laycok *et al*., 1991). Application of adjuvants in the formulation of herbicide sometimes cause up to 100% mortality of target weed within 48 hours (Winder and Watson, 1994). Yang and Jong (1995) prepared an inverted emulsion formulation of *Myrothecium verrucaria* by mixing and aqueous spore suspension with oil phase (1:1 v/v), where only oil emulsion carrier killed the 7 weed plants species. Auld (1993) developed an oil suspension emulsion formulation for control of *Xanthium spinosum*. Spores of *Colletotrichum orbicularae* were mixed with Kaolin (aluminium silicate powder) and dried. Dried powder (200 mg) was mixed with 20 mL of vegetable oils, 2 mL of an emulsifier and the water added to a volume of 200 mL. This formulation was tested in the field by Klein *et al.* (1995) and got up to 99% mortality in the first year. Two bioherbicides Collego and BioMal were commercially available as a wetable powder (Boyette *et al*., 1996). This formulation involves the drying of spores harvested from liquid fermentation, together with a carrier such as Kaolin which can be stored before suspension in water (Norman and Trujillo, 1995; Klein and Auld, 1996).

Solid formulation: Fungal pathogen infect weeds at or below the soil are best studied to solid of granular formulations (Connick, 1988; Boyette *et al*., 1991) which may consist of grains, peat, charcoal, clay, vermiculite, alginate, bagasse, mineral soil or filter mud as carrier (Green *et al*., 1998). These formulations of bio herbicides are better suited to pre emergence applications, attacking weed seedlings as they emerge from the soil (Connick, 1988). Since granular formulations contain dried propagules they may have a longer shelf life than liquid based formulations and is very important for a commercial bioherbicide (Auld, 1992).

During the last two decades, increasing interest has been observed on the synthetic beads of various materials for immobilization of herbicides, microorganism, cells and enzymes, antibodies, animal embryos and artificial seeds (Kierstan and Bucket, 1977; Barrett, 1978; Connick, 1982; Banerjee *et al*., 1984; Bashan, 1986; Cosby and Dukelow, 1990; Ling-Fong *et al*., 1993; Kremer *et al*., 2006). Biodegradable slow release beads comprised of sodium alginate and skim milk were developed as carriers for the bacterial inoculation of plants (Bashan, 1986). Walker (1981) developed a granular formulation of *Alterneria macrospora* for control of *Anoda cristata*. Mycellium of the pathogen was grown in liquid formulation, mixed with the horticultural vermiculite, exposed to diurnal light for 24 h to allow sporulation and air dried for 24-48 h. Field application of the granular inoculums incited almost 100% infection of *A. cristata* giving 75-95% control.

CONSTRAINTS IN BIOHERBICIDE DEVELOPMENT

In spite of considerable research in bioherbicides, there are only two commercially available products in North America in 1996 (Mortensen, 1998) due to the constraints in bioherbicide development. Host variability

Table 4: Methods, advantage and constraints of controlling parasitic weeds (Sauerborn *et al*., 2007)

Method	Advantage	Constraints
Quarantine	Cheap	Regulatory constraints
Crop rotation	Cheap	Not effective, seeds remain dormant
Trap crop	Cheap	Not feasible to flush out very large number of seeds
Classical breeding	Non-polluting	Resistance in short term (3-6 years)
Soil fumigation	Non-selective	Too expensive, regulatory constraints
Herbicide	Cheap	Phytotoxic to crop
Resistance inducer	Cheap	not yet known
Micro-organisms	Cheap	Regulatory constraints, expensive with identification, isolation and formulation
Insects	Environmental friendly	Regulatory constraints, expensive with identification, isolation and formulation
Solarization	Non-selective	Expensive
Herbicide resistant crop	Weed control as well	Fraught with risk, resistance in short term (3-6 years)

and host range is the main biological constraints of bioherbicide development (Gabriel, 1991). Sub-optimal temperature, moisture and compatibility with other pesticides are probably the most important environmental constraints for the efficacy of foliar bioherbicide (TeBeest, 1991; Kelly *et al*., 2006) in the field application. Formulation of a bioherbicide agent is one of the most challenging technological constrains to the development of reliable and efficacious product (Mortensen, 1998), as mass production of viable, infective and genetically stable propaguales of plant pathogens is a major requirement (Boyette *et al*., 1996). Sauerborn *et al*. (2007) described the major advantages and constraints of the parasitic agricultural weeds (Table 4). Due to these constraints several efficient bioherbicide agents have not been developed for commercial use by industries because of their low market potential and high cost of production (Charudattan, 1991; Templeton, 1992; Heap *et al*., 1993; Mortensen, 1996; Ghosheh, 2005).

CONCLUSIONS

With the development of sustainable agriculture and consciousness of human environmental protection government and enterprises will pay more attention to the study of exploitation of microbial pesticides because of their potential benefit for the environment (Li *et al*., 2003; Yandoc *et al*., 2004; Boyetchco and Rosskof, 2006). More than 27 exotic plant pathogens have been investigated for classical biocontrol of weeds and 67 weeds have been targeted using at least 107 fungal taxa as bioherbicide agents and 18 weed species have been targeted using deleterious rhizobacteria (Mortensen, 1998). From these literatures it can assume to be good potential of microbial weed control in future as the use of microbial herbicide will increase tremendously by 20% every year (Li *et al*., 2003, Turnera *et al*., 2007). It is necessary to overcome the constrains in this approach. New pesticide based on biosynthesis and molecular modification by gene technology would be the integrative steps for the exploitation of potential microbial herbicides.

Myrotheceium verrucaria was first evaluated for sickletod (Walker and Tilley, 1997) and kudzu control (Boyette *et al*., 2002) since, it has been evaluated for multiple targeted weeds. Multiple-Pathogen Strategy is a novel approach to increase the level of control of the targeted weed (Charudattan, 2001). Anderson and Hallett (2004) observed that culture filtrate from *M. verrucaria* had broad-spectrum activities across many plant families. Hallett (2005) points out that there are opportunities for the development of bioherbicides for some specialized niches, such as parasitic, urban and allergenic weed. Development of broad-spectrum bioherbicide not only for a particular species but also for a weed community of specific agricultural field or crop is highly demandable (Mahanta *et al*., 2007). Although most biological control agents are too host specific to individually address mixed weed population in agronomic field crops, they can be targeted to manage those weeds that have the maximum impact on crop yield in high valued crops where control options are limited.

Hoagland (1996) presented several approaches for improving biocontrol efficacy by disrupting the target weeds defense mechanism, including the use of herbicide or other compounds that affect key enzymes, blocking the synthesis of secondary plant metabolites or breaking down physical berries (Hodgson *et al*., 1988; Gressel *et al*., 2002; Peng and Byer, 2005; Hirase *et al*., 2006). Tiourebev *et al*. (2000) have attempted a novel approach to enhancing virulence of weed biological control agent by selecting for strains that capable of excreting high levels of amino acids that can suppress the growth and development of plants causing leaf distortion, loss of apical dominant and stunted growth. Development of multi-combination formulation and commercially available products of bioherbicide would be the potential approach for the successful weed management in future.

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