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DISCOVERY AND DEVELOPMENT OF MYCOHERBICIDE FOR BIOLOGICAL CONTROL OF *Parthenium hysterophorus*: OPPORTUNITIES & FUTURE NEEDS

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ABSTRACT

Weed management through chemical herbicides creates spray drift hazards and adversely affects the environment. Besides, herbicide residues in food commodities, directly or indirectly, affect human health. These effects lead to the search for an alternate method of weed management which is eco-friendly. In this regard, biological approaches are gaining momentum. They include a high degree of specificity to target weed, with no effect on non-target and beneficial plants or man, absence of weed resistance development, and absence of residue build-up in the environment. Currently, fungal weed control is rapidly developing natural phenomena in research areas with implications for plant yield and food production. Fungal weed control may help to maintain the quality of crops and reduce the use of chemical pesticides and other toxic chemicals and offer important natural mortality factors for weed population control under natural environmental conditions. The application of the fungal spores, fermented broth, and their crude metabolite or purified metabolites is a very good source for natural herbicide for the management of Parthenium weed. Fungal weed pathogens can produce a wide array of toxins, bioactive metabolites with different biological activities, chemical structures, mechanisms of action, specificity with respect to plants, and environmental impact and stability. This paper will discuss the current research progress on fungi and their secondary metabolite application for the management of Parthenium weed.

INTRODUCTION

Parthenium hysterophorus Linnaeus (Asteraceae), globally known as feverfew, ragweed or Parthenium is a weed of world significance. It is most popularly known as 'congress grass' throughout India while in Hindi speaking belt known by the popular name of 'gajar ghas'(carrot grass). It degrades natural ecosystems by reducing biodiversity (Holm *et al*. 1997) and can cause serious allergic reactions in man and animals (Chippendale and Panetta 1994, Sushilkumar 2012). In India, it has invaded almost all types of crops and has become a serious threat for agricultural production. Sushilkumar and Varshney (2010) estimated infestation of Parthenium in 18.78, 14.25 and 2.0 Mha lands in barren, fallow, wasteland including land under non-agricultural uses, crop area under cultivation and forest areas, respectively. In India, this weed is a serious problem in states like, Andhra Pradesh, Bihar, Haryana, Karnataka, Madhya Pradesh, Tamil Nadu and Uttar Pradesh. Parthenium is regarded as one of the worst weeds because of its immense capacity of reproduction and ability to thrive in varied climatic conditions. Its low photorespiration under arid conditions, photo and thermo-insensitivity, C3/C4 intermediate mechanism, more biomass production at elevated atmospheric CO2 conc. compared to the normal in a rapidly changing climate make it more invasive (Pandey *et al*. 2003, Naidu and Paroha 2008, Tang *et al*. 2009, Naidu 2013, Sushilkumar 2014). Now, Parthenium has invaded about 35 Mha of land throughout India (Sushilkumar and Varshney 2010). After being established in India, Parthenium has gradually spread into most of its neighbouring countries like Pakistan (Shabbir and Bajwa 2006), Sri Lanka (Jayasurya 2005), Bangladesh (Rahman *et al*. 2008, Karim 2009) and Nepal (Adhikari and Tiwari 2004, Shrestha *et al*. 2014).

Global Strategies used for Management

Parthenium hysterophorus has showed a negative impact on the natural and agro ecosystem, it is necessary to manage this weed before it sets seed and continues to spread. The several methods has used for managing this weed. These include physical or mechanical, chemical and biological control methods. The following outlines are given some of the

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successful and potential best management practices used throughout the India and the rest of the world.

Manual Methods

Manual methods of weed control have earlier been considered as one of the most effective way to eradicate weeds. However, Industrialization has resulted in severe labour shortage and drastic increase in labour cost has significantly hampered this method. Manual removal of *P. hysterophorus* by hand weeding before flowering and seed setting is the most effective method, but it is not necessarily practical or economical particularly where there are large infestations. This method, however, may pose a health hazard from allergic reactions and a danger that mature seeds will drop and increase the area of infestation. Other mechanical treatments, such as grading, mowing, slashing and ploughing are also considered inappropriate since they may also promote seed spread as well as rapid regeneration from lateral shoots close to the ground (Panse *et al*., 2014; Shrinivas *et al*., 2014). Ploughing the weed before the plants reach the flowering stage may be effective. Although burning is not promoted as a control strategy, it has been used to control the first flush of emergent weeds at the beginning of the rains in Australia but is only considered a short-term control measure (Kumar *et al*., 2014). Burning has been shown to create open niches in the landscape, into which larger number of *Parthenium* seeds are able to germinate in the absence of vegetation.

Chemical Methods

Synthetic chemical herbicides has no doubt played very crucial role in weed management since 1960s, however, due to indiscriminate and excessive use of these chemicals, several problems have arisen. Contamination of ground water, accumulation of residues, development of resistance, narrow spectrum of activity, injury to non target organisms, lack of residual effectiveness etc, are the major public concern nowadays. It is important that *P. hysterophorous* be sprayed early before flowering and seed set. Farmers should scout their fields regularly to check for escaped or untreated isolated infestation. Vegetable farmers prefer a rapid knock-down of weeds before they plant or do cultural control and work towards weed-free plots. Repeated spraying may be required even within a single growing season to prevent further seed production. In this regard, their approach is to use a single herbicide with a broad-spectrum application with the intention to rid the field of grasses, broadleaves and sedges. The most commonly used herbicide over the last 70 years in the various countries has been paraquat and diquat. These herbicides work very well and usually give very quick control of most weeds, but sometimes cause severe drift damage. It has been accepted that the overuse of this chemical over the years, in addition to the *P. hysterophorous* metabolic pathways (Bridgemohan & Brathwaite, 1987; Brathwaite, 1978; Bridgemohan, 2012) has developed resistance to paraquat and has established the predominance of the weed. This apparent resistance, coupled with the high reproductive capacity of the weed and its wide-ecological amplitude, has given rise to the increased scourge of *P. hysterophorous* in vegetable crops. In other parts of the world, a similar response is obtained with respect to the resistance by both herbicides. Therefore, there is a need to discover and develop new, economically and environmentally sustainable weed management technology.

Biological Control

Bio-control of *P. hysterophorus* is reported as the most cost-effective, environmentally friendly and ecologically viable method of control. While several organisms exist locally, there is no observable damage to either seedlings or mature plants. Now, much emphasis has been given to control Parthenium through various biological agents like pathogens, insects and plants**.** Biological control is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of natural enemies. There are three basic strategies to implement the biological control of weeds which is mentioned below.

Classical Approach

By insect:- The basis of the classical approach is the introduction of natural enemies from which the exotic weed has escaped and relies on self-sustaining epidemics of introduced organisms to control the target weed at acceptable levels (Huffaker, 1957; Wapshere, 1982; Watson, 1991). The vast majority of agents used in classical biological control have been phytophagous insect (Wapshere *et al*., 1989). Several insects have been tried to control Parthenium weed in the different countries viz., the leaf-feeding beetle (Zygogramma bicolorata) and the stem galling moth (Epiblema strenuana), both imported from Mexico, have shown good potential to control this weed. The beetle, Z. bicolorata, an effective leaf eater, was imported from Mexico for the management of Parthenium in Australia in 1980, and in Indian Institute of Horticulture Research (IIHR) (Parmelee, 1967). Both the adults and larvae of this insect feed on leaves. The early stage larvae feed on the terminal and auxiliary buds and move on to the leaf blades as they grow. The fully-grown larvae enter the soil and pupate. An insect density of one adult per plant caused skeletonization of leaves within 4–8 weeks but little success has been achieved as the weed has very high generative potential, and moreover the insect is not a species specific and is found to attack sunflower in India (Jayanth, 1987).

By Rust fungi: In standard classical biological control strategy, obligate parasites, especially rust fungi, are the first choice because they exhibit narrow host ranges, high reproductive capacities, and efficient aerial dispersal (Tomley, 2000). The most promising fungal agents to manage parthenium are Puccinia abrupta var. partheniicola, Puccinia xanthii var. parthenii-hysterophorae, Entyloma compositarum De Bary (Ustilaginales), and Plasmopara halstedii (Farlow) Berl. and De Toni (Peronosporales). Of these, Puccinia abrupta var. partheniicola and Puccinia xanthii var. parthenii-hysterophorae originate from Mexico and have been fully screened and released in Australia; they are the most potential classical bio-control fungal pathogens of this weed in Australia.

The Mycoherbicidal approach

The term 'Mycoherbicide' was introduced by Daniel *et al*. (1973), who stated that an endemic i.e. native pathogen might be rendered completely destructive to its weed host by applying a massive dose of inoculum at a particular susceptible stage of weed growth. It would shorten the lag period of the inoculum build up and pathogen distribution essential for natural epiphytotics. The term 'Mycoherbicide' has been redefined as plant pathogenic fungi developed and used in an inundative strategy to control weeds in the way chemical herbicides are used (TeBeest & Templeton, 1985) or as a living product that controls specific weeds in agriculture as effectively as chemicals (Templeton *et al*, 1986). The technical difficulty of producing obligate parasite at large scale is also a deterrent to their use as mycoherbicide. Thus, the fungi that are either facultative parasitic or facultative saprophyte, highly virulent, host specific and genetically stable but constraint naturally by low inoculum production and poor dissemination are probably the best candidate for development as mycoherbicide (Templeton *et al*. 1986ab; Templeton & Heiny, 1989). Prasad (1993) stated that an ideal mycoherbicide must have attitude of growing readily in artificial culture media and of producing abundant and durable spores or other reproductive structure readily infects its target host under a variety of environmental conditions and causing mortality or reducing competition within a short period of time. The product should also have a reasonable long storage life and be compatible with ingredient of tank mixes. Finally, the bioherbicide should be search that can be easily adapted to common application technology and must be cost effective in order to compete with chemical herbicides. Various aspects of assessment of efficacy of mycoherbicidal candidate have been elaborately discussed in many publications (Charudattan, 1989, 91; Charudattan *et al*., 1990; Gupta, 1998; Templeton *et al*., 1986 ab, Templeton & Heiny, 1989; Tisdell & Auld, 1989).

To date, two fungal plant pathogens have been registered as bioherbicide weed control products in the United States and one registered in Canada. DeVine·, a liquid formulation of Phytophthora palmivora (Butler) Butler was registered in 1981 for control of Stanglervine (Morrenia odorata (H. & A.) Lindl.) in Florida citrus graves (Ridings, 1986). COLLEGO·, a dry power formulation of Colletotrichum gloeosporioides (Penz.) Sace. f.sp. aeschynomene was registered in 1982 for the control of northern jointveteh (*Aeschynomene virginica* (L) B.S.P.) in rice and soybean in Arkansas, Louisiana and Mississippi (TeBeest & Templeton, 1985). BioMal, a dry formulation of Colletorichum gloeosporioidesf.sp. malvae wasregistered in 1992 in Canada for the control ofround-leaved mallow (Mava pusma Smith.) in wheat and lentils (Mortensen, 1988; Makowski & Mortensen, 1992). Another product, Lubao 1 Sn (*Colletotrichum gloeosporioides* f.sp. *cuscutae*), is being used in China for the control of dodder (*Cuscuta chinensis* and *C. austrais*) on soybean (Wan *et al*, 1994).

Host specificity is another important consideration in the bioherbicide approach (Watson. 1985). However. Optimization of spore production ("fermentation") and formulation and application of a bioherbicide product are often critical aspects in determining the success or failure of a bioherbicide prospect (Watson & Wymore. 1990; Boyene *et al*. 1991).

Biological control of weeds with plant pathogenic fungi offers opportunities for overcoming several of these inadequacies as evidenced by commercialization of many strains of fungi as mycoherbicides (Aneja, 1998; Auld, 1990; Bhan *et al*., 1998; Boyette & Abbas, 1995; Pandey, 1999, 2000; Pandey *et al*.1995-2003;Kovics *et al*., 2005).

Biorational Approach

Phytotoxins derived from pathogens and other microorganisms are also useful for weed control (Duke, 1986; Hoagland, 1990; Strobel *et al*., 1992). Traditionally. research on phytotoxins has been limited to products produced by plant pathogens of crop plants. These phytotoxins have proven useful as tools for screening plants for toxin insensitivity (disease resistance) and as probes of normal physiological plant functions (Strobel *et al*., 1992). Weed pathogens have had a long period to coevolve with their hosts and devise biochemical mechanisms to weaken them or influence their gross physiology (Strobel, 1982; Strobel *et al*., 1992). Hence, there is the potential 10 use natural compounds produced by plant pathogens as herbicides or to utilize them as building blocks for novel herbicides (Duke, 1986; Duke & Lydon, 1987; Kenfield *et al*., 1989; Kennedy *et al*., 1991). Herbicidal activity has been demonstrated in many species of the actinomycetes *Streptomyces* genus. and two herbicides, NK-ü49 (methoxyphenone) and bialophos have been developed from microbial metabolites (Duke, 1986; Watson, 1993). Other microbial toxins, including maculosin, a host-specific phytotoxin for spotted knapweed (*Centaurea maculosa* Lam.), isolated from liquid cultures of *Alternaria alternata* (Fr.) Keissier (Stierle *et al*., 1988) and fumonisin BI, a broader-spectrum phytotoxin isolated from *Fusarium moniliforme* Sheldon (Abbas & Boyette, 1992), have been suggested 10 have utility in weed control (Watson, 1993).
We have made significant progress in the development and application of mycoherbicide for weed *Parthenium hysterophorus*. Significant research and development efforts over a long period, have led to several successful case studies that have provided great impact in mycoherbicide control of weeds Parthenium. A series of surveys have been carried out to search for naturally occurring fungal pathogens on parthenium to control it through the bioherbicidal strategy. The severity of pathogen to the reproductive organs led to serious damages of the Parthenium plants and may be used as a potential mycoherbicide against this weed (Singh, 2007). Weed pathogens are able to produce a wide array of toxins, bioactive metabolites with different biological activities, chemical structures, mechanism of action, and specificity with respect to plants, environmental impact and stability. Keys to the development of biologically-based agents such as mycoherbicides and phytotoxins as effective and practical components of weed management systems are the advancement of practical, reliable, cost-effective methods for their production, stabilization, formulation, and application. Some of the advantages of mycoherbicides over traditional chemical herbicides are their specificity for the target weed; absence of adverse effects on humans, wildlife or domestic animals; rapid degradation and absence of residues in surface or ground water, crops, soil or food chains. The optimal growth and maximum phytotoxin production condition of isolates were investigated as well. Many aspects relating to these fungi, e.g., characterization, production process, recovery, formulation, factors affecting infectivity were also evaluated. Such positive results from the tested biopesticides pushed this study to recommend further studies on their effective use against this weed.

Opportunities & Future Needs

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 market because the cost of developing them may be less than that for a chemical herbicide. Production technology ins 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 1988; 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, Cercospora rodamanii, 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 as major areas of importance:

More mycoherbicide candidates of important weeds- With each weed- pathogen system,, new conceptual and practical problems are bound to come to light. These in turn will provide a deeper understanding of mycoherbicides.

Integration of mycoherbicides with chemical pesticides- As an on going effort, the compatibility- incompatibility of mycoherbicides and chemicals should continue. This will be mandated by the fact that each weed –mycoherbicide- pest management system will be different and specific recommendations for the use of mycoherbicides will be needed.

Integration of mycoherbicides and chemical plant growth regulators for improved weed control through decrease in weed growth and increase in mycoherbicide efficacy- Weeds possessing high raters of vegetative growth and vegetative proliferation tend to be difficult to control with mycoherbicides. The ability to outgrow disease pressure 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).

Extensive survey of literature- It indicates that the role of weed pathogen interaction in weed management have neglected significantly. More knowledge is needed in this area alongwith phytoalexin production, defense protein etc. Researches in these areas would definitely help in weed management.

Development of suitable formulations 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 the adverse 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).

Fermentation 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 air–lift 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.

1. 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.
2. Increased public and private funding as well as administrative support for research and development of mycoherbicides.
3. 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 may be 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.

CONCLUSION

Due to its invasive capacity and allelopathic properties, P. hysterophorous has the potential to disrupt the natural ecosystem and threaten the biodiversity. From an earlier survey, the authors concluded that under systems of intensive vegetable production and where the use of paraquat and glyphosate are widespread, the weed has shown the ability to survive herbicide treatments, except at the seedling stage, regardless of the season and crop or management practices. In addition, biological and cultural control were insignificant in reducing Parthenium 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.

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