Chapter 11

Bioherbicides

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Abstract

Bioherbicides are biologically based control agents useful for biological weed control. Hence, bioherbicides have been identified as a significant biological control strategy. Bioherbicides have many advantages such as clearly defined for target weeds, no side effect on beneficial plants or human health, a lack of pesticide residue build-up in the environment, and effectiveness for control of some herbicide-resistant weed biotypes. More importantly, it has been demonstrated that mixtures of some bioherbicides and synthetic herbicides can be more effective. Apart from many bioherbicide benefits, some factors have been noted to restrict the development of bioherbicides into profitable products. They involved environmental, biological and technical–commercial restrictions.

Keywords: Bioherbicide (inundative) approach, advantages, restrictions

1. Introduction

Development of alternative weed control methods is needed to help decrease reliance on herbicide use. Biological weed control is an alternative option for weed problems, particularly in agriculture and forestry. It is based on the use of natural enemies, particularly insects and pathogens to control weeds, as a sustainable, low cost and more environmentally acceptable method of weed control. One of the approaches to biological weed control using pathogens, mainly fungi, is inundative, bioherbicide approach.

Bioherbicides are phytopathogenic microorganisms or microbial phytotoxins useful for biological weed control applied in similar ways to conventional herbicides [1–3]. The active ingredient in a bioherbicide is, however, a living microorganism. Most commonly the organism is a fungus; hence the term mycoherbicide is often used in these cases [4]. Although the use of fungi and bacteria as inundative biological control agents (bioherbicides) has been recognized as a significant technological weed control alternative [5–9], it can be argued that it serves a more important role as a complementary component in successful integrated management strategies [10], and not as a replacement for chemical herbicides and other weed management tactics [11]. Actually, in many situations, bioherbicides can be used as the sole option for the management of one or two target weeds, i.e. as a minor supplement to conventional chemical herbicides [12].

However, according to many authors, bioherbicides offer many advantages in comparison with synthetic herbicides. They include:

- a high degree of specificity of target weed;
- no effect on non-target and beneficial plants or man;
- absence of residue build-up in the environment; and
- effectiveness for managing herbicide-resistant (HR) weed populations [7,9,12–16].

Except numerous advantages of bioherbicides, some circumstances have been noted to restrain the progress of bioherbicides into profitable outputs. These include:

- biological restrictions (host changeability, host scope resistance mechanisms and interaction with other microorganisms that affect efficacy) [17];
- environment restrictions (epidemiology of bioherbicides reliant on optimal environmental conditions) [18–20];
- technical restrictions (wholesale production and formulation development of reliable and effective bioherbicide) [17,21]; and
- commercial restrictions (market capacity, patent protection, confidence and adjustment) [21–23].

2. Biological weed control

Biological control is the deliberate use of natural enemies to reduce the population of a target weed to below a desired threshold [24,25] and can be divided into two main approaches:

1. classical approach, in which a natural enemy is exported from its native range to an introduced (weedy, invasive) range of a plant, [24,26,27], and
2. bioherbicide approach, in which a natural enemy is used within its native range to control a native or naturalized weed [28–30].

2.1. Classical (inoculative) approach

The biocontrol approach using an imported pathogen to control a native or naturalized weed with minimal manipulations has been termed the inoculative or classical biocontrol method [31]. The classical approach is directed mainly towards the control of exotic weeds, which have

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spread in the introduced area in the absence of natural enemies. Control is achieved by the importation and release of highly host-specific pathogens virulent to the target weed in its native region [32]. These agents feed on the weed, reproduce and gradually suppress the weed as their population grows.

A highly successful biological control programme was implemented in Hawaii in the 1970s when a white smut fungus (*Entyloma ageratinae* sp. nov.) was introduced from Jamaica to control the exotic weed mistflower (*Ageratina riparia* (Regel) K. & R.), which was invading Hawaiian indigenous forest. The effect was rapid, with 95 per cent control after 3–4 years [33]. Western Australian golden wattle (*Acacia saligna* (Labill.) H.L. Wendl.) is regarded as the most important invasive weed that threatens the Cape Fynbos Floristic Region of South Africa, a unique ecosystem. In about 8 years, the introduction of the rust disease (*Uromycladium tepperianum* Sacc.) from Australia had become widespread in the province and tree density was decreased by 90–95% [34].

Another widely acclaimed example of biological control success is the use of a rust fungus (*Puccinia chondrillina* Bubak & Syd.) to control rush skeleton (*Chondrilla juncea* L.) in Australia. The rust fungus was introduced from the Mediterranean. *Puccinia chondrillina* was also introduced into the western United States to control a *Chondrilla juncea* L. biotype. However, unlike in Australia, the rust was only partially successful [35]. An example of successful classical biological control is that of rust fungus (*Puccinia carduorum* Jacky), imported from Turkey and released into the northeastern United States (Virginia and Maryland) in 1987 to control musk thistle (*Carduus thoermeri* Weinm.). The rust fungus has spread widely from its original introduction to the western states of Wyoming and California [36–38]. Baudoin *et al.* [36] found that *Puccinia carduorum* reduces musk thistle density by rushing agedness of rust-infected musk thistle and diminishes seed production by 20– 57%. Rust fungus (*Puccinia jaceae* var. *solstitialis*), imported from Bulgaria and Turkey, was released in California, United States, in 2003 for biological control of yellow starthistle (*Centaurea solstitialis* L.). The host range tests on this pathogen were extensive [39,40].

Trujillo *et al.* [41] have introduced septoria leaf spot (*Septoria passiflorae* Syd.) for the biological control of the exotic weed banana poka (*Passiflora tripartita* (Juss.) Poir var. *tripartita* Holm-Nie. Jörg. & LAW), at different forest sites in Hawaii, which resulted in over 50 per cent biomass reduction of the weed 3 years after inoculation.

Klamathweed beetle (*Chrysolina quadrigemina* Suffrian), introduced from Australia, proved especially effective for common St. Johnswort (*Hypericum perforatum* L.) weed control on California rangeland. Populations of the beetles quickly grew and spread. After 5 years, millions were collected from original release sites for redistribution throughout the Pacific Northwest. Ten years after the first release, H. perforatum populations in California were reduced to less than 1% of their original size [25]. Another example of successful classical biological control is the introduction of the black dot spurge beetle (*Aphthona nigriscutis* Foudras) from Hungary as a biological control agent for leafy spurge (*Euphorbia esula* L.). Release of these insects has resulted in a 99 per cent reduction in spurge stand density in one area and a corresponding 30-fold increase in grass biomass in pasture and rangeland after 4 years [42,43].

2.2. Bioherbicide (inundative) approach

Opposite to classical (inoculative) approach, the bioherbicide (inundative) approach uses indigenous plant pathogens that are isolated from weeds and are cultured to produce large quantity of infective material [28]. These are utilized at amounts that will provoke tremendous levels of infection, leading to elimination of the target weed before economic damages happen [29]. A development of this strategy involves application of weed pathogens in a manner similar to herbicide applications. Bioherbicide inoculum is susceptible to unfavourable environmental conditions after spraying, and viability needs to be maintained for as long as is necessary to achieve infection following application [30]. Once in the field, the inundative application of inoculum is timed to coincide with the most favourable environmental conditions and susceptible growth stage of the weed, so that a disease epidemic occurs and the weed population is suppressed [44,45]. Once the weed problem has been removed, natural constraints ensure that the pathogen population returns to a low level once again.

The bioherbicide (inundative) approach has been successfully implemented for a number of important agricultural, invasive and exotic weeds. Many examples dedicated to positive bioherbicide implementation are elaborated in the Section “Bioherbicide case studies”.

3. History of bioherbicides

Utilization of plant pathogens for weed control was first reported in the early 1900s, but the concept of using bioherbicides to control weeds attracted wide interest among weed scientists and plant pathologists after the Second World War. The earliest experiments simply involved fungus *Fusarium oxysporum* Schlecht. against prickly pear cactus (*Opuntia ficus-indica* (L.) Mill.) in Hawaii. In the 1950s, the Russians mass-produced the spores of *Alternaria cuscutacidae* Rudakov and applied them to the parasitic weed dodder (*Cuscata* spp.). In 1963, the Chinese mass-produced a different fungus (*Colletotrichum gloeosporioides* f. sp. *cuscutae*) for the same weed (*Cuscata* spp.). They called their mycoherbicide “LuBao” and an improved formulation is still in use today. Official date of bioherbicide control of weeds commenced in the late 1960s with an ambitious programme to find out a pathogen or pathogens for sorrels or docks (*Rumex* spp.) in the United States [46] and blackberries (*Rubus* spp.) in Chile [47]. From the 1970s there has been a considerable number of prosperous bioherbicide projects [8,24,48]. The number of scientific articles on bioherbicide research has enlarged excessively since the early 1980s. The number of weeds aimed for control as well as the number of potential pathogen candidates studied has increased. Registered and unregistered uses of bioherbicides have also increased considerably. In addition, the numbers of US patents published for the bioherbicidal technology and bioherbicide handling have increased, perhaps anticipating an increased dependence on bioherbicides in the future [49].

4. Bioherbicide case studies

Considering the research effort expended in this area, some bioherbicides are commercialized (Devine®, Collego®, BioMal®, Camperico®, Myco–TechTM®, Woad Warrior®, Smolder®, Dr. bioSedge®, Biochon®, StampOut® [13,22,28,50–55] and many are underway to develop and register. Plant pathologists and weed scientists have identified approximately 200 plant pathogens that are candidates for development as commercial bioherbicides [48,56]. Some examples are presented below.

Culture filtrates of *Plectosporium tabacinum* (van Beyma) M. E. Palm, W. Gams et Nirenberg, isolated from naturally infected cleavers plants, provided 80–90% control of *Galium* spp. under field conditions. [57]. *Fusarium oxysporum* (PSM 197), a potential mycoherbicide for controlling *Striga* spp. in West Africa, showed significant reductions in the total number of emerged plants of *S. asiatica* (91.3%), *S. gesneroides* (81.8%) and *S. hermonthica* (94.3%) [58]. Hemp sesbania (*Sesbania exaltata* [Raf.] Rydb. ex A. W. Hill), one of the 10 most troublesome weeds in soybean in Arkansas, Louisiana and Mississippi [59] was 90% controlled with the isolates of the fungus *Colletotrichum truncatum* [14–16]. The level of control was similar to those achieved with the synthetic herbicide acifluorfen in the same crop [15]. A *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar:Fr. (MV) bioherbicidal isolate IMI 361690 provided >85% control of *Chenopodium amaranticolor* Coste & Reynier, *Senna obtusifulia* L., *Sesbania exaltata* (Raf.) Cory and *Datura stramonium* L. [60]. Other MV isolates have bioherbicidal activity for the control of *Carduus acanthoides* L. and *Euphorbia esula* L. [61,62]. Trichothecenes produced by an MV isolate from Italy could inhibit seed germination of the parasitic plant *Orobanche ramosa* [63]. Recently, MV was shown to be highly virulent against *Portulaca oleracea, Portulaca portulacastrum, Euphorbia maculata* and *Euphorbia prostrata* in commercial tomato (*Lycopersicon esculentum* L.) fields in the southeastern United States [64]. *Phomopsis amaranthicola*, an indigenous plant pathogen, provided up to 100% control of several *Amaranthus* species [65–67]. Host range testing of this organism has not shown infection of soybean, corn, sorghum or wheat. Mintz *et al*. [68] evaluated another fungal pathogen, *Aposphaeria amaranthi* Ell. & Barth. (later renamed as *Microsphaeropsis amaranthi* (Ell. & Barth.) [69], as a potential bioherbicide for several *Amaranthus* species (*A. retroflexus, A. spinosus*, *A. hybridus* and *A. albus*). In this context, in field experiments, eight *Amaranthus* species treated with *Microsphaeropsis amaranthi* and a mixture of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* had severe disease ratings of 15 days after treatment (DAT), and mortality ranged from 74% to 100% [70]. *Drechslera avenacea* is a potential bioherbicide for *Avena fatua* control in dryland wheat crops in southern Australia. Maximum disease severity (DS) (1.1 lesions per mm^2 of leaf tissue) was recorded following the application of 1×10^5 spores per mL and exposure of weeds to a 12- to 16-h dew period at 20–25°C [71]. The fungus *Pyricularia setariae* applied at the concentration of 10^5 spores mL^{-1} reduced fresh weight of *Setaria viridis* (L.) Beauv. by 34% 7 DAT when compared with controls, whereas a concentration of 10^7 spores mL^{-1} reduced fresh weight by 87%. More importantly, *Pyricularia setariae* caused 80% fresh weight reduction of *Setaria viridis* (L.) Beauv. biotype resistant to sethoxydim, compared with 17% achieved with sethoxydim [72]. *Sesbania exaltata* [Raf.] Rydb. ex A.W. Hill was effectively controlled by 85, 90 and 93% of *Colletotrichum truncatum* (Schwein.) Andrus & Moore at inoculum concentrations of 2.5, 5.0 and 10.0 x 10^6 spores mL^{-1}, respectively

[73]. *Taraxacum officinale* was controlled by 70–80% and 90% by biocontrol strains of *Phoma macrostoma* applied as granular fungal inoculums to soil at the rate of $63g/m^2$ and $125g/m^2$, respectively [74]. The fungus *Phoma macrostoma* exhibits control of broadleaved weeds *Taraxacum officinale* and *Cirsium arvense* while showing no effect on grasses or cereal crops and is now being developed as a biological herbicide for weeds in turfgrass (lawns, golf courses, public grounds), agriculture (cereal crops) and agro-forestry (reforestation nurseries) [75]. Kadir *et al*. [76] have demonstrated the efficacy of *Dactylaria higginsii* as a bioherbicide agent for *Cyperus rotundus* L. in field trials. They have also reported that *Dactylaria higginsii* disease could help reduce interference from *Cyperus rotundus* L. and improve yield in greenhouse-grown tomato [77]. Morales-Payan *et al*. [78] estimated the bioherbicidal efficacy of *Dactylaria higginsii* in several field trials in Florida and Puerto Rico. According to their results, application of *Dactylaria higginsii* at 8 and 18 days after emergence (DAE) or 8, 18 and 25 DAE reduced the yield of pepper to 24 and 31%, respectively, compared to weed-free control plots. Similarly, Semidey *et al*. [79] have reported that onion yield was higher in plots sprayed three times with *Dactylaria higginsii* as compared to the yield from one or two applications. The potential of *Dactylaria higginsii* as a substitute to methyl bromide fumigation in an integrated approach to *Cyperus rotundus* L. control in a tomato production system was examined by Rosskopf *et al*. [80]. The results obtained showed that weed seedlings between 3 and 5 weeks of age were the most susceptible to the disease. Besides the use of fungi as bioherbicides, several strains of soil bacteria as pre-emergent biological control agents against annual grassy weeds have been identified and field-tested. Up to 85–90% control of green foxtail (*Setaria viridis* (L.) P. Beauv.) and wild oat (*Avena fatua* L.) was achieved using a granular formulation called "pesta" [81–83]. The leading bacterial candidate for biological control of the grass weeds is a *Pseudomonas fluorescens*, strain BRG100, which delays the emergence of the weeds and significantly inhibits root growth. Charudattan *et al*. [84] reported on potential virus-based bioherbicide tobacco mild green mosaic virus (TMGMV), which caused 83–97% mortality of *Solanum viarum* plants of different sizes and ages.

5. Interaction between bioherbicides and synthetic herbicides

The idea of combining bioherbicides with synthetic herbicides or adjuvants has been the issue of substantial research work. Moreover, it has been revealed that mixtures of some bioherbicides and synthetic herbicides can be synergistic [85,86], culminating from reduced weed defence reactions caused by the herbicides, consequently making the weeds more sensitive to pathogen attack [87,88]. Christy *et al*. [86] reported a synergy between trimethylsulfonium salt of glyphosate and *Xanthomonas campestris* against several weed species. Other synergistic interactions involving chemical herbicides and bioherbicides have been discovered and some were granted patents in the United States [85,89]. According to Caulder and Stowell [85,89], acifluorfen and bentazon were the most effective synergists and provided significant control in several weed/pathogen combinations: (*Senna obtusifolia*, formerly *Cassia obtusifolia* [L.] Irwin & Barneby) and *Alternaria cassiae* Jurair & Khan; *Aeschynomene virginica* [L.] Britton, Sterns & Poggenb. and *Colletotrichum gloesporioides*; *Sesbania exaltata* (Raf.) Cory and *Colletotrichum*

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truncatum; and *Desmodium tortuosum* [SW.] DC. and *Fusarium lateritium* Nees. A sublethal dose of glyphosate (50 mmol L^{-1}) suppressed the biosynthesis of a phytoalexin derived from the shikimate pathway in Senna obtusifolia (L.) H. S. Irwin & Barneby, infected by *Alternaria cassiae* Jurair & Khan, reducing the resistance of the weed to fungal infection and disease development [90]. Similarly, 12 DAT, *Brunnichia ovata* [Walt.] Shinners and *Campsis radicans* [L.] Seem. ex Bureau were controlled by 88 and 90%, respectively, through a synergistic interaction between the fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar: Fr. and the herbicide glyphosate. Neither glyphosate nor *M. verrucaria* controlled these weeds at commercially acceptable levels (≥80%) [73]. According to Boyette *et al*. [91], timing of glyphosate application in relation to combined treatment with the bioherbicide *M. verrucaria* can improve the control of *Pueraria lobata* (Willd.) Ohwi, *Brunnichia ovata* [Walt.] Shinners and *Campsis radicans* [L.] Seem. ex Bureau. Heiny [92] revealed that *Phoma proboscis* Heiny at 1 x 10^7 spores mL^{-1} mixured with reduced rates of 2,4-D plus MCPP controlled field bindweed (*Convolvulus arvensis* L.) more effectively than the herbicide mixture alone and as effectively as the pathogen at a 10-fold higher rate. Application of various crop oils [68,93–97] and invert emulsions [15,98–100] improved efficacy and performance of many bioherbicides and biocontrol fungi. For instance, according to Hoagland *et al*. [10] treatment of fungus *Myrothecium verrucaria* (MV) strain originally isolated from sicklepod (*Senna obtusifolia* L.) mixture with the surfactant Silwet L-77 caused 100% mortality of *Pueraria lobata* (Willd.) Ohwi seedlings under greenhouse conditions, and 90–100% control of older *Pueraria lobata* (Willd.) Ohwi plants in naturally infested and experimental plots, respectively.

6. Bioherbicide limitations

In spite of considerable research in bioherbicides, there are only a few commercially available products worldwide. This lack of availability is mainly due to limitations in bioherbicide development, which need to be overcome to ensure the future commercial success of bioherbicides [22, 101]. Limitations in bioherbicide development can be classified as either environmental (temperature and, particularly, humidity as major factors influencing the efficacy of bioherbicides), biological (mainly host variability and resistance), or technological–commercial (mass production and formulation, which often blocked bioherbicide development) [17,22,102].

7. Environmental limitations

Environmental limitations are a constraint to the effective use of many biological agents, including bioherbicides. Environmental factors influence formulation performance of bioherbicides as inoculum production is dependent on sporulation of the formulation. This process, although rapid, might continue over several weeks subsequent to applications and might encounter variable environmental conditions [18,21,22]. In the application of bioherbicides, environmental conditions prevailing in the phyllosphere of plants are frequently hostile for biological control agents [103,104]. A requirement of more than 12 h of dew period for severe infection by a pathogen has been reported for several potential bioherbicides [105–108] and this may limit the efficacy of the bioherbicide in the field. Temperature generally has not been considered to be as critical as moisture for mycoherbicide [109], although field efficacy of *Colletotrichum orbiculare* in controlling *Xanthium spinosum* L. is reduced by high-temperature conditions after inoculation of plants [110]. However, dew period length requirement and temperature typically interact [111]. Low temperatures may greatly extend dew period length requirements for bioherbicides developed for use in crops, such as winter wheat.

Nutrient balance can play an important part in sporulation of fungi. Studies with *Colletotrichum truncatum* have shown how carbon concentration and carbon to nitrogen (C:N) ratio influence propagule production [112]. Moreover, a defined amino acid composition of the N source improved the production of conidia [113]. In addition, spore fitness in terms of germination and appressoria formation rate and subsequent disease production [114] was influenced by C:N ratios.

Soil environment, moisture and the nutrient status of the soil can influence the physiology of target plants and, therefore, their interaction with aerial applied bioherbicides [21]. Pre-emergence application has been considered as an alternative approach to overcome some of the environmental stresses imposed upon propagules applied onto the foliage or soil surface [115]. Bioherbicides consisting of propagules of soil-borne pathogens, which normally infect at or below the soil surface, appear to be more protected from environmental extremes and may persist and give residual control [116,117]. In this context, Jackson et al. [113] reported for 95% control of the emerging *Sesbania exaltata* (Raf.) Rydb. ex A. W. Hill seedlings when *Colletotrichum truncatum* (Schw.) Andrus and Moore was incorporated into the soil.

There are many environmental limitations to applying bioherbicides and maintaining their efficacy in water as well [118]. Auld and McRae [4] stated that for control of aquatic weeds a biocontrol agent would need to possess a high ecological capability to contend with varying conditions between surface and bottom, as well as across even small bodies of water. Oxygen concentration, temperature, light intensity and salinity are just four of the variables to contend with.

8. Biological limitations

From a biological viewpoint, a good bioherbicide acts relatively quickly and has acceptable efficacy in control of weeds. Unfortunately, Charudattan [8] stated that many of the discovered weed pathogens may provide partial control of only one weed species, even under ideal conditions. This host particularity is related to the fundamental bio-physiology of the pathogen and to host changeability [119,120] and resistance as well [17]. In other words, within a population of weed species there will usually be a range of genetically diverse biotypes [121] that may include some resistant biotypes, just as there may be a range of biotypes of microorganisms [122], for instance within fungal species, with slightly different host ranges [14,123,124], so that there is potential to mix and vary the biotypes of a species used as a bioherbicide. Non-target plant protection in relation to the potential use of *Chondrostereum purpureum* (Pers ex Fr.) Pouzar (silverleaf disease) to control black cherry (*Prunus serotina* Erhr.) in coniferous forests by modelling the dispersal of spores and therefore quantitatively assessing the risks to susceptible fruit trees outside the forest was noted by De Jong *et al*. [125]. Concerns have been raised regarding the potential for sexual or asexual gene exchange between bioherbicide strains and strains attacking distantly related crop plants [109,126,127].

9. Technological–commercial limitations

Several technological limitations have been identified that could prevent the widespread use of bioherbicides [21]. Pathogenic strains, formulation method and the interaction of these two parameters significantly affect the shelf life of the formulations at room temperature [21,128]. High concentrations and the alteration of formulations are needed to increase bioherbicide activity [129]. Compatibility testing of formulation components that range from registered agricultural products to novel substances, such as sunscreens, humectants and starches, can consume a great deal of time and resources [130].

The most challenging aspect of formulating bioherbicides is to overcome the dew requirement that exists for several of them. Attempts to overcome this limitation have included developing various water-retaining materials; invert and vegetable oil emulsion formulations [15,94,131] and granular pre-emergence formulations [132] are considered as a promising approach to make pathogens less dependent on available water for initial infections to occur [133,134]. In addition, appropriate formulations can also reduce the dosage of inoculum required to kill weeds [135], thus potentially reducing the cost of bioherbicides.

Experiments conducted with a number of potential bioherbicides have demonstrated that an invert emulsion allowed infection to occur in the absence of available water [15,133,136] and reduced the need to apply high dosages of inoculum [135]. Invert emulsions consist of a continuous oil phase that contains water droplets. Connick and Boyette [137] have developed an invert emulsion formulation exhibiting lower viscosity and greater water-retention properties. Auld [93] reported that application of low concentrations of vegetable oils with an emulsifying adjuvant enhances efficacy of *Colletotrichum orbiculare* in inciting disease on *Xanthium spinosum* L. in the absence of dew in greenhouse conditions. However, according to the same author, oil emulsions were not effective in the field conditions. An invert emulsion has been shown to overcome dew requirements and reduce the spore concentrations required [15]. But, unfavourable characteristic is containing of more than 30% oil which makes these formulations expensive and very viscous, typically requiring special spraying equipment such as air-assist nozzles, and because of the high oil content it is likely to produce phytotoxic effects on non-target plants [135,138]. Invert emulsions have been shown to cause phytotoxicity in some cases and to predispose a variety of plants to opportunistic pathogens as well [99].

From the other side, the main restriction in the application of solid (dry) forms of bioherbicide is that they must await suitable, moist conditions for fungal growth and infection [139].

Moreover, during this waiting period the living active ingredients must survive in the field. In addition, ant theft has been a problem with some formulations [140].

The simplest liquid formulations of bioherbicides are water suspensions of spores often with a small amount of wetting agent. These are generally used as standards against which to compare more complex formulations. However, under ideal conditions for fungal infection, simple aqueous suspensions can be successful in the field [110]. Pathogenicity of an aqueous mycelial inoculum of *Alternaria eichhorneae* Nag Raj & Ponnapa in a controlled environment experiment was improved with hydrophilic polymers such as gellan gum, alginates and the polyacrylamide [141]. Although several polymers retained considerably more water after 6–8 h than the water-suspension controls, no increase in efficacy of the fungus *Colletotrichum orbiculare* was found [142]. Vegetable oil emulsions that contain 10% oil and 1% of an emulsifying agent reduced dew dependence in controlled environment studies using *C. orbiculare* in control of *Xanthium spinosum* L. [93]. Unfortunately, in the field conditions, the efficacy of these formulations was variable [143].

A novel bioherbicide formulation uses a complex emulsion – water-in-oil-in-water (WOW) emulsion [144]. It contains at least one lipophilic surfactant, at least one hydrophilic surfactant, oil and water. Although used in the pharmaceutical [145], cosmetic [146] and food industries [147], WOW emulsions do not appear to have been widely used in agricultural or horticultural technology. Although numerous improvements of liquid formulations of bioherbicides have been made, genetic manipulation of fungi offers a broad extent of opportunities to adjust formulations and to ameliorate bioherbicide characteristics [148].

Taking into account the above-mentioned restrictions, the production of bioherbicides by profit-oriented companies would involve additional expenditure without guaranteed income. The amount of abundant development and production of phytopathogenic microorganisms or their phytotoxins for bioherbicides in immerse or in solid-state systems, which would alter from one bioherbicide to another, is relatively high [149]. In addition, the small market capacity of considerable competent bioherbicide aspirants reveals that market capacity could be a restraint for developing such herbicides. Because of that, firms are suspicious that development and registration expenditures will be paid back [21,22].

10. Conclusion

The bioherbicide access to weed control is attaining impetus. New bioherbicides will be applicable in inundate lands, badlands as well as in control of parasite weeds or HR weeds. Research on synergism between pathogens and herbicides for their incorporation in effective weed management, applied science, fungal metabolites and biotechnology utilization, principally genetic engineering is needed. Bioherbicides will not deal with all of the environmental and weed control issues related with synthetic herbicides, nor will they alter the present or future depository of synthetic herbicides. To a certain degree, their appearance will presumably be complementary components in lucrative weed management systems, and in the revelation of different phytotoxins with new performances and new molecular sites of

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action. Advanced research on this field is imperative in order to entirely find out mutual interactions of phytopathogenic microorganisms, crops and weeds, and to identify new plant pathogens or their phytotoxins promising effective for the new-generation bioherbicides.

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