Review

Microbial agents for control of aquatic weeds and their role in integrated management

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 Received:
 14 May 2012

 Accepted:
 15 October 2012

doi: 10.1079/PAVSNNR20128014

The electronic version of this article is the definitive one. It is located here: http://www.cabi.org/cabreviews

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Abstract

Aquatic ecosystems throughout the world are threatened by the presence of invasive aquatic plants, both floating and submerged. Some of the aquatic species, such as water hyacinth (*Eichhornia crassipes* [Mart.] Solms), alligator weed, *Alternanthera philoxeroides* (Mart.), giant salvinia, *Salvinia molesta* D.S. Mitchell and water lettuce (*Pistia stratiotes* L.), Griseb. despite being relatively minor problems in their native range, have become major invaders of aquatic habitats in other parts of the world after having escaped from their natural enemies. Unchecked growth of aquatic vegetation is generally undesirable and reduces the value of the water resource. Despite adopting all control options including manual, mechanical, chemical and classical biological, the problem persists. The current weed management is oriented towards finding approaches that are effective in controlling the weed and reducing environmental contamination from herbicides. Plant pathogens have been gaining increasing attention and interest among those concerned with developing environmentally friendly, effective and compatible approaches for integrated management of the noxious weeds. This paper discusses some of the major microbial agents associated with aquatic weed sand their increasing role in integrated weed management.

Keywords: Aquatic weeds, Biological control, Mycoherbicides.

Introduction

Aquatic plants grow and complete their life cycle in water. Some of them, after coming out of their native range, grow aggressively, causing significant ecological impacts on the environment, and associated cascading socioeconomic effects causing harm to aquatic environment, directly or indirectly, and attain the status of a weed [1]. These invasive plants are largely anthropogenically spread, and their presence is typically an indication of the enrichment of waters through pollution, as a result of increasing urbanization, industries and agriculture [2]. Most of the world's worst tropical aquatic weeds are native to the neotropics, a vast biogeographic area comprising of South and Central America, the Caribbean and parts of Southern Mexico. Some of the species, such as water hyacinth, Eichhornia crassipes (Mart.) Solms, alligator weed, Alternanthera philoxeroides (Mart.) Griseb., giant salvinia, Salvinia molesta D.S. Mitchell and water lettuce,

Pistia stratiotes L., despite being a relatively minor problem in their native range, have become major invaders of aquatic habitat in other regions of the world after having escaped from their natural enemies [3].

The presence of unwarranted aquatic vegetation influences the management of water in reservoirs, manmade canals, river systems and natural waterways, which amount to millions of kilometres/square kilometres of such water bodies around the world [4, 5]. Dense impenetrable infestations restrict access to water, often reduce the usefulness of aquatic bodies for pisciculture [6] and related commercial activities, the use of irrigation canals, navigation and transport, hydroelectric programmes and tourism [7–9]. They greatly increase water loss through evapotranspiration when they completely cover the surface of a water body [10] and decrease light penetration, which affects the diversity and population of native aquatic flora and fauna in these habitats [11–12]. Aquatic weeds can assimilate large quantities of nutrients

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from the water reducing their availability and quality. Thus they pose a grave threat to native flora and fauna and seriously deplete water bodies of oxygen [13, 14]. Poverty-stricken rural populations whose livelihoods depend on access to clean freshwater waterways are the most negatively impacted. Dense growth of aquatic weeds may provide ideal habitat for the development of mosquito causing malaria and other vectors for diseases such as encephalitis, filariasis, bilharzia and cholera [15, 16]. In addition, weeds may devalue riverine real estate [17, 18]. Aquatic weeds have been found to severely reduce the flow capacity of irrigation canals thereby reducing the availability of water to the agricultural fields. The flow of water is reduced by 40-95 percent and retardation coefficient increases from 0.024 to 0.055 in irrigation channel [19, 20]. In paddy fields, water hyacinth has been reported to interfere with seed germination and seedling establishment, resulting in heavy economic losses of up to US\$24 million [20]. The dense growth of aquatic weeds may also interfere with navigation, damage pumps and turbines in superthermal and hydroelectric power stations, affecting electricity production and increasing the cost of maintenance of power stations. The red water fern, Azolla filiculoides Lam. 1783 (Azollaceae), alone and often together with Lemna minuta Kunth, L. minor (Thuill. ex P. Beauv.) A. Chev. and Spirodela polyrhiza (L.) Schleiden, settles in ponds, ditches, water reservoirs, channels and slow-flowing rivers, forming dense mats. These mats of floating plants can affect the aquatic habitat by eliminating submerged plants and algae [21], preventing their photosynthesis and blocking oxygen diffusion. For these reasons, invasive aquatic plant infestations need to be controlled to mitigate their negative impacts on ecosystems, livelihoods and economies.

There are several control mechanisms that have been implemented for preventing the spread of, or eradication of, aquatic weeds, which include physical (harvesting, water-level fluctuation, sediment alteration, nutrient limitation and light alteration), chemical (herbicides) and biological control (using living organisms, such as insects, nematodes, bacteria or fungi) strategies. Each has its benefits and drawbacks. Manual and mechanical control methods are used widely but they are not suitable for large infestations and are generally regarded as a shortterm solution [22]. Although chemical control methods are available that offer quick solution to the unwanted vegetation, they have their own limitations because of their non-target environmental impact [23-26]. There are other aspects such as toxicity to fish and other forms of life in the aquatic habitat, the deterioration of water quality from persistent chemical and dispersal of toxic chemicals through food chain [23, 27]. Such environmental concerns have fuelled the upsurge of interest in biological control of aquatic weeds, which is considered a cost-effective, permanent and environmentally friendly method. Biological control of weeds with plant pathogens is an effective, safe, selective and practical means of weed

management that has gained considerable importance over the last five decades. They are considered to be the most effective alternatives to chemical herbicides [28]. DeVine, developed by Abbott Laboratories, USA, was the first commercial mycoherbicide derived from fungi *Phytophthora palmivora* Butl., a facultative parasite that produces lethal root and collar rot of its host plant *Morrenia odorata* H. and A. Lindl. (strangler wine) and persists in soil saprophytically for extended period giving a long-term control [29]. The use of plant pathogenic fungi as biological control agent has often been very successful against various exotic aquatic and terrestrial weeds.

This paper reviews various aspects of biological control using micro-organisms and their concepts and applications in integrated management of aquatic weed and proposes novel integrated weed-control strategies.

Bioherbicides

In the last few decades, bioherbicides have gained considerable importance. These organisms offer considerable scope as potential agents against several weeds [30–32]. They have relatively critical application times and suppress, rather than eliminate, a pest population. They have limited field persistence and a short shelf life and present no residue problems, so are safer to humans and the environment than conventional pesticides [33]. Success stories of these products and the expectation of obtaining perfect analogues of chemical herbicides have opened new routes for aquatic weed management.

Microbial herbicides as inundative biocontrol agent

Fungal agents formulated as 'mycoherbicide' are projected as an environmentally benign replacement for chemical herbicides in weed infested areas [34, 35]. Development of microbial herbicides can be especially beneficial against the herbicide-resistant weeds. Commercial mycoherbicides first appeared in the US market in the early 1980s with the release of the products called DeVine, Collego and BioMal [36]. Early research and field success of bioherbicides, particularly mycoherbicides, suggested that a large number of spores or mycelial mass of native, targetspecific fungi could be used to turn a normally endemic pathogen into an epidemic [37]. The use of phytopathogenic fungi as biological control agents for aquatic weed species has increased the global attention during the last few decades. Several workers [38-43] undertook field surveys to neotropics in search of promising exotic pathogens.

Among all aquatic weeds, fungal pathogens associated with water hyacinth have been extensively studied. Among the promising pathogens of water hyacinth are *Alternaria eichhorniae* Naj Raj & Ponnappa, *Acremonium* zonatum (Sawada) W. Gams, Cercospora piaropi Tharp. (=C. rodmanii Conway; [44]), Myrothecium roridum Tode, Rhizoctonia solani J. G. Kühn, Uredo eichhorniae Gonz. Frag. and Cif., etc [45, 46]. C. piaropi was associated with rapid and widespread decline of water hyacinth in the Rodman Reservoir, Florida [47]. Thus it was extensively studied [48, 49], patented by the University of Florida, USA and has been commercially developed as mycoherbicide against water hyacinth under the trade name ABG-5003 by the Abbott Laboratories of USA [36, 50]. Field testing of the bioherbicide failed to produce desired results, and additional development of the product never followed [51]. A. eichhorniae has been extensively studied for biocontrol potential against water hyacinth [52-54]. It has been shown to be reasonably host-specific to water hyacinth [52, 55] and capable of severely damaging and suppressing this weed [55–57]. However, among several problems, in developing it as a potential mycoherbicide, one of the major obstacles to the use of A. eichhorniae as a mycoherbicide for water hyacinth is its requirement for at least 10 h of dew to allow the applied inoculum to germinate and infect and, to an extent, to colonize the weed [55]. The rust fungus U. eichhorniae, field recorded only in South America, despite showing great potential against water hyacinth [41], could not be released in the field because all life stages of the rust had not been documented [32]. A. zonatum was widely studied by Rintz [58] from a classical biocontrol perspective. It was concluded that the pathogen did not seem capable of killing water hyacinth or seriously deter their prolific growth in USA and it did not appear to cause significant damage. However, more virulent strains are reported to occur in Mexico [40]; so better strains may be obtained in future from other water hyacinth-infested areas. Despite this rich microbial biodiversity, no practical microbial herbicide has been developed thus far other than C. piaropi for water hyacinth.

It is surprising that not much work has been done to study the plant pathogens of *S. molesta*, another aquatic weed of great significance. Possibly the great success that resulted from the introduction of *Cyrtobagous salviniae* Calder and Sands has reduced the need for studying additional agents of the weed [59]. However, several preliminary studies [60–64] have brought into some potential pathogens such as *Phoma glomerata* (Cda) Wollenw. and Hochapf., *Nigrospora sphaerica* (Sacc.) Mason, *M. roridum* Tode ex Fries, *R. solani* Kühn and *Verticillium nigrescens* Pethybridge as potential pathogens of salvinia species.

Barreto and Torres [65] reported two pathogenic fungi, Nimbya alternantherae (Holcomb and Antonopoulos) Simmons and Alcorn and Cercospora alternantherae Ellis and Langlois as potential fungi on alligator weed, A. philoxeroides (Mart.) Griseb. Cercospora pistiae Nag Raj, Govindu and Thirumalacharand and Cercospora canescens Ellis and Martin have been reported on P. stratioites from various parts of the world [66].

Surveys for pathogens of hydrilla, Hydrilla verticillata (L.f.) Royle and Eurasian water milfoil, Myriophyllum spicatum L. with classical biological control potential were carried out in the 1990s in Asia and Europe [67-69]. Although the biological control potential of several promising isolates from these surveys has been evaluated [67, 70], further studies are needed to demonstrate the safety and efficacy of these pathogens. Fungal pathogens against several other aquatic weeds including Fusarium culmorum (Wm.G.Sm.) Sacc., Plectosporium tabacinum (J.F.H. Beyma) M.E. Palm, W. Gams & Nirenberg and Mycoleptodiscus terrestris (Gerdemann) Ostazeski against hydrilla [71, 73] have been considered. Studies by Smither-Kopperl et al. [72, 74] showed that under laboratory conditions, P. tabacinum was highly pathogenic to hydrilla shoots maintained in aqueous solutions in test tubes. Infected shoots became slightly chlorotic within 24 h and the leaves became flaccid. There was also an increase in disease severity as inoculum concentration increased. M. terrestris has been extensively studied as a bioherbicide for hydrilla management singly and in combination with herbicides [73, 75–77]. An aggressive isolate of M. terrestris was reported from the surveys conducted in Texas during the 1990s, which demonstrated the excellent potential for development as a bioherbicide [77]. In developing *M. terrestris* as a bioherbicide, emphasis has been placed on the production of microsclerotia that are melanized, compact hyphal aggregates that may survive desiccation and serve as the over wintering structure for the fungus rather than thin-walled spores (conidia) or hyphal units [77]. In a liquid broth culture medium, microsclerotia can be induced to develop over a 4-day fermentation period [77]. The microsclerotial propagules are then harvested through a dewatering process, air dried to moisture content of 5-10%, vacuum packed and stored at 4°C. A prototype formulation of *M. terrestris*, positively tested for efficacy against dioecious hydrilla was further produced by Trans America Product Technology, Inc. (St. Charles, MO), by incorporating the fungus into a patented biocarrier, Biocar[®] 405. Initial test tube studies demonstrated that both granular and caplet formulations induced severe disease on excised hydrilla shoot tissue at two weeks post inoculation. Low, medium and high dosage rates of the granular formulation applied to rooted hydrilla in 12 litre columns reduced shoot biomass at 4 weeks post-application by 87.7, 94.8 and 99.2%, respectively, compared with untreated controls [78]. In tank studies, a granular formulation reduced shoot biomass of hydrilla grown in 1700 litre tanks by 97.5% at 4 weeks post-application [78, 79]. However, initial field trials of *M*. terrestris formulated with Biocar[®] 405 failed because the company changed the ingredients in the carrier that inadvertently killed the fungus [80, 81].

The submerged aquatic weeds, *Egeria densa* Planchón and *E. najas* Planchón are reported to be severely damaged by *Fusarium graminearum* Schw. The plants developed progressive chlorosis, followed by necrosis and complete tissue disintegration, after being exposed to inoculum of this isolate. A series of *in vitro* and *in vivo* studies that were carried out resulted in a possible product provisionally called FUSGRA [59].

Morris et al. [82] recorded the occurrence of Xanthomonas campestris (Pammel) Dawson causing a bacterial disease of parrot feather, Myriophyllum aquaticum (Vell.) Verdc, in South Africa. The disease was characterized by the wilting and greying of scattered, individual aerial shoots from the tip downward for about 10 cm. Microscopic examination revealed that the xylem vessels of the stems and leaves were filled with X. campestris cells. Although natural infections seldom caused more than 1% of the aerial shoots to be affected, an inundative application of the bacterium at 10⁸ colony-forming units (cfu)/ml produced 100% shoot infection when the plants were sprayed in the morning when guttation droplets were still present on the leaves [82]. Although all aerial parts of the plant were dead, about 6 weeks later new shoots appeared from the submersed stems and the plants recovered. Examination revealed that the bacterium did not invade the older underwater stems. Because of this inability to kill submersed biomass and the ability of the plant to replace killed shoots, the bacterium was not considered an effective bioherbicidal agent [82] unless integrated with other control options.

Several authors [83–85] studied pathogens associated with *M. spicatum*, but none of them had potential to be effective under field conditions. Often under greenhouse conditions, many weeds can be easily killed with mycoherbicidal agents applied at high doses. In the natural environment or field conditions, their evolutionary balance allowed weed populations to withstand most pathogen attacks because of their genetic heterogeneity.

Phytotoxic metabolites from micro-organisms as bioherbicide

Plant pathogenic fungi are one of the most effective biologically based alternatives to chemical herbicides but several ecological constraints are associated with them as most pathogens require environmental parameters to be met before infection or symptoms of disease can occur. In many instances, environmental constraints, such as adverse temperature, soil or water pH and humidity are responsible for reduced disease incidence and severity [86]. Furthermore, environmental conditions are everchanging, and are difficult to predict or duplicate growthchamber studies. To overcome these problems attention is focusing on the secondary metabolites produced by the pathogens. Microbes have been a profitable source of phytotoxins with the potential to lead to new herbicides [87]. Several workers [88–90] isolated a toxin from A. eichhorniae and obtained leaf necrosis on water hyacinth. Metabolites of a fungal pathogen of hydrilla have shown phytotoxicity against the weed [91]. More such

studies are required to be undertaken to develop potential mycoherbicides for aquatic weeds.

Mycoherbicides in Integrated Management of Aquatic Weeds

It is expected that an inundative application of pathogen would overwhelm plant defence mechanisms resulting in a disease epidemic and reduction in biomass similar to that achieved with the use of herbicides. For several weeds such as salvinia and *Azolla*, the use of single insect biological control agent has been sufficient to effect control but novel approaches are required for the control of some of the other aquatic weeds such as water hyacinth [3]. Integrated management of weeds is a holistic approach aimed at minimizing weed impact while simultaneously maintaining the integrity of the ecosystem. The integration of several techniques reduces the reliance on any single control technique.

There is evidence that combinations of treatments can be more effective for controlling several weeds than individual treatments [92, 93]. There have been reports on vectoring of pathogenic fungi by insects [94-96]. Several insect biocontrol agents have also played an important role in spread of phytopathogens of a specific weed [97-100]. Interactions between arthropods and several saprophytic and parasitic fungi and bacteria are common on arthropod-damaged water hyacinth [101]. The efficacy of A. zonatum and C. piaropi were significantly enhanced when applied to water hyacinth in presence of Neochetina weevils [102, 103]. When feeding, weevils made holes in the leaves that allowed the fungi to penetrate. During some of our recent studies, we found that disease index of some of the potential phytopathogens of water hyacinth in South Africa was significantly higher on Neochetina-damaged water hyacinth as compared with undamaged plants [92].

Further, combined treatment using two- and threepathogen combinations have also resulted in causing larger lesion diameters on water hyacinth than any of the pathogens tested singly [93]. Templeton and Heiny [104] suggested that several isolates of one pathogen or several species of pathogens each having slightly different environmental requirements could be mixed in the formulation to ensure that at least one would encounter the optimal environmental window. Hasan and Ayers [105] reported that interaction between the biotroph and necrotroph occurs at the infection site of biotrophs, where infection by one pathogen makes the host more susceptible to secondary infection. The synergistic relationship of two pathogens can provide biological and economical feasibility by the use of the mixtures of two or more fungi for effective control of one or more weeds. Den Breeyen [106] while conducting similar study reported greater lesion diameters on water hyacinth when using combination of pathogens than lesion diameter using individual pathogens only.

Integrating sub-lethal doses of chemical herbicides, phytotoxins from plants or microbes or growth retardants with fungal pathogens is a promising technology that will play an important role as an alternative aquatic plant management tool. Charudattan [107] attempted to integrate a microbial herbicide, natural population of arthropods and chemical herbicides. He suggested a judicious combination of chemical and biological control over time and space may help reduce the water hyacinth management costs by improving management efficiency. Ray *et al.* [26] recommended integration of herbicide glyphosate at low doses with the insect and fungal biocontrol agents. However, at higher concentration the herbicides can have detrimental effect on the biocontrol agents.

Integrated weed management practices have long remained unnoticed as an approach for controlling submersed aquatic weeds. The discovery of herbicideresistant hydrilla in several lakes in Florida has elevated the need to identify new technologies that minimize recurring use of chemicals [108]. Recent studies have shown that combining the indigenous fungal pathogen, M. terrestris, with low doses of herbicides has excellent potential as an integrated strategy for long-term management of hydrilla while reducing the risk of damage to desirable, non-target species [75-77]. Netherland and Shearer [76] found applying sublethal doses $(2 \mu g/l)$ of fluridone with either 100 or 200 cfu/ml of M. terrestris reduced hydrilla biomass more than 90%, and was more efficacious than applying either of the control agents alone. Studies by Nelson and Shearer [108, 109] on integrating fungi and herbicide to control Eurasian water milfoil using *M. terrestris* and the herbicide 2,4-D resulted in better weed control compared with either of the agents used independently. Sorsa et al. [110] demonstrated that combining low levels (0.65-1.29 ppm) of endothall with the fungal pathogen Colletotrichum gloeosporioides (Penz.) (Penz. & Sacc.) significantly enhanced control of Eurasian water milfoil. Additional treatment benefits included reduced chemical input into the environment, longer-term weed control, and increased selectivity as a result of lower herbicide use rates.

A successful implementation of integrated weed management programmes requires long-term planning, knowledge of the biology and life cycle of the weed and the appropriate control methods. It is also important to recognize the strengths and limitations of each control techniques and to integrate the appropriate technique in time and space to achieve the best result.

Future Trends of Mycoherbicides in Aquatic Weed Control

Although enormous efforts have been made to control aquatic weeds in the past few decades, several of them are still problematic in various parts of the world. It is a challenge to develop an effective bioherbicide that is

acceptable for use in practical weed management programmes. During the last two decades, the society has experienced a growing interest in organic farming and eco-friendly approaches of pest management, partly as a result of the growing public awareness about environmental degradation and contamination of soil and water. Researchers and policy makers are becoming conscious of the short-term benefits of using chemical herbicides and long-term positive effects of the use of mycoherbicides and other ecologically safe methods to deal with noxious weeds. Future weed management technologies will take on novel manifestations improving microbial herbicide technology with an aim to eliminate or greatly reduce the use of chemical herbicides. Such improvement can only be brought about by renewed research on improving the potential of existing microbial agents of weeds with research on improving their formulation (including mass culturing, adjuvant and shelf life), application technologies and also perhaps using tools of biotechnology and genetic engineering. For example, Tiourebev et al. [111] have attempted a new approach to enhance virulence of a microbial agent by selecting strains that are capable of producing high levels of amino acids that can suppress the growth and development of plants causing leaf distortion, loss of apical dominance and stunted growth. Charudattan et al. [112] altered the virulence and host range of a bacterium by inserting genes, which encoded for the production of bialaphos, a glutamine-synthetase inhibiting herbicide. Alteration of such bacterial genes could produce overall increases in virulence, host range or other related traits. Transfer of genes controlling toxin production or specific enzymes to improve mycoherbicide performance is also an important arena for development [113]. Similarly, it would be possible to remove undesirable characters of a plant pathogen using standard genetic engineering practices. For example, a mammalian toxin gene could be removed or disrupted. Today, virtually any heritable trait of a bioherbicide can be enhanced or suppressed using techniques of genetic engineering [114]. Molecular data can clarify taxonomy and evolutionary relationships, and uncover evidence of closely related species that cannot be morphologically distinguished. Thus microherbicides for management of aquatic weeds hold great potential in the near future.

Acknowledgements

The Working for Water Programme and Rhodes University, Grahamstown, South Africa, are acknowledged for their financial assistance to this project.

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