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Review Article

Microbial Phytotoxins as Sources of Herbicides: Current Development and Future Scope

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Abstract

Weeds are a problem in a variety of land uses, associated with declines in crop yields, quality and health nuisance. The herbicide resistance and chemical pesticides are the major issue for new generation due to strong support for developing a new novel compound to control weeds. Due to less user of chemical herbicide in current scenario, it is very important to develop a new class of bioherbicide with novel mode of action which is not previously developed. Development of ecofriendly weed control has shown a novel and innovative path to Scientist for developing a natural herbicide based on microbes. It is known that microorganism produce thousands of secondary products, many of which are phytotoxic and may potentially be used as herbicides or templates for the synthesis of new herbicides. The interest of researchers is therefore focused particularly on pathogen living on weeds. Microbial products are attractive candidate for potential use in agriculture. They are characterized by highly specific activity and high selectivity while at the same time they are readily biodegradable. They belong to very diverse groups such as polyketides, terpenoids, diketopiperasines, isocoumarins etc. of course, these compounds are too complex in structure to be used herbicide. However, secondary products of microorganism represent subject matter of wide research and supplement the organic synthesis in the development and extension of new biologically national and cost-effective herbicides. The potential microbial marasmin to control some noxious important weeds is reviewed here. The noxious weeds are *Parthenium hysterophorus, Lantana camara, Xanthium strumarum, Cassia tora, Hyptis suveolens, Sida actua* and *Antigonon leptopus*. It is concluded that of this weeds, best suited as targets for biological control using microbial metabolites.

Keywords: Microbial Herbicides; Noxious Weeds; Current Status; Future Perspectives

Introduction

A Microbial herbicide is a microbes-based control agent for weeds. They are made up of microorganisms (e.g. bacteria, viruses, fungi) that can target very specific weeds. The invasive genes of microorganisms can attack the defense genes of the weeds and kills it. Microbial herbicide are natural compounds of fungi, bacteria, actinomycetes or protozoa. A bioherbicide based on a fungus is called a mycoherbicide. In the industry, bioherbicides and other biopesticides are often referred to as "naturals". The application of biobased products to achieving this goal has received increasingly great attention over the last three decades. Biorational strategy has lot of advantage over chemical herbicide, it is ecofriendly, target specificity, less production costs compared to chemical herbicides and they have novel herbicidal mechanisms. The development of phytotoxins as weed biocontrol agents has been reviewed by various Scientist [1-3]. Natural product of microbes provides good source of novel compounds for control of weeds. Some of the microbial phytotoxins are bialaphos produced by *Streptomyces viridochromogenens* and *S. hygroscopicus* [4]. The Bialaphos is a non-selective phytotoxin phosphinothricin and commercialized in the name of herbicide glufosinate. Its mode of action site is glutamine synthetase (GS). With similar mode of action, there are several natural products of microbes but they are not effective like glufosinate as a viable herbicide [5,6]. The microbial natural product having variation in host specificity, some are host specific and some have no specificity are known as non host specific [2,7,8]. Sometime nonspecific microbial toxins are advantage over host specific because they have potential to kill various range of weeds without phytotoxicity to crops [9]. The tentoxin (a cyclic tetrapeptide) which is produced by several *Alternaria* species and causes severe chlorosis in many of the problem species associated with soybeans and maize without affecting either crop is the best example for non host specific toxin [10]. Only a small proportion of potentially useful microbial metabolites have been described herein, but examination of the structures leads to at least four conclusions. The first most example for this is a fermentation products having diverse features and possess unique control nature. The second most example are specific classes of compounds contain congeners that have dissimilar biological activity. The third example are some synthetic changes may be made to alter the biological properties of natural products without, apparently, destroying the bio gradable properties. The fourth properties is biologically active natural products offer unique and novel template for synthetic work to pesticide industry. They have lot of beneficial structures for the future development in microbial world and some are yet to develop. They are biodegradable in nature and microbes based herbicide will be on the great market in future generation [1,4,6].

Present status

Microbial Herbicides are part of modern agricultural production offer a cost effective, efficient and appropriate manner of weed control contributing considerably to production increase and stability. However synthetic herbicides have unfavorable toxicological characteristics, persistence and a potential environmental impact. The development of resistance and high costs has contributed to develop a new approach [11]. On the other hand, bearing in mind side effects of pesticide on human health (chronic toxic effects of many compounds, reproduction, impact, mutagens, oncogenic effect etc.) and contaminating residues in water and soil. The problems caused by excessive use of pesticide in conventional agriculture have initiated the development of a sustainable agriculture concept with special focus on alternative hazardous organism control in agroecosystems through implementation of biological control. Namely as it is generally known microorganism produce hundreds of thousands of secondary metabolites products many of which are phytotoxic and many potentially be used as herbicides or templates for the biosynthesis of new herbicides. These compounds are very diverse in structure, ranging from simple to complex molecules having very different molecular weights [10]. Advantages of natural products of microbes over synthetic herbicides:

- Investigations can be conducted with high investments
- No risk to human health and environments
- Highly selective toward plant species
- Registration of natural compounds is cheaper
- Shorter half life.

Microbial herbicide prototype models and expectations

There have been many articles reported by researchers on successful microbial agents with bioherbicidal potential, this has created the optimistic impression that additional bioherbicides are close to becoming commercially available. Also, the expectation of weed mortality using terminology such as Microbial herbi-"cides" has generated the illusion that these organisms have or must possess identical features to chemical herbicides and will lead to the eradication or near-kill of weed populations [12,13]. The fact that biological herbicides are not analogues to chemicals, lacking in some of the features of chemicals, has perhaps led to the opinion by some critics that this technology has failed to deliver the goods. But is this truly the case when in fact we are comparing the traits and benefits of biologically-based technology using the paradigm of chemically-based technology? The first generation of bioherbicides had a variety of characteristics that were comparable to those of chemical herbicides and deemed to be desirable for their commercial success [14-16]. Successful bioherbicides were expected to provide high efficacy, often resulting in high weed mortality. Other traits considered in early bioherbicide development were host-specificity (i.e. preference for narrow host-range), ease of use, genetic stability, cost-effective mass production, and ability to provide rapid weed control with predictable field performance. While these characteristics are attractive, many biological herbicide candidates have fallen short of meeting these requirements. Both Collego® and DeVine® were considered great achievements because they provided at least 90% weed control that was effective and consistent [17]. However, their efficacy on single weed species has limited their commercial success, particularly when one considers that most agroecosystems are comprised of multi-species weed communities. It may be difficult to justify for many farmers to use or for industry to market a single product to control a single target weed. In addition, these two products have targeted weeds with specialized markets and limited profit margins [18]. On the other hand, a product such as Camperico®, with a single economic target, has a high value market in the golf course industry [17]. The development of *Chondrostereum purpureum* as a mycoherbicide has expanded its utility because of its broad-spectrum activity on a variety of woody tree species such as red alder (*Alnus rubra*), black cherry (*Prunus serotina*), white birch (*Betula papyrifera*), and aspen (*Populus* spp.). Other examples of bioherbicide candidates exhibiting a broad host-range include *S. minor* for dandelion and other broadleaved weed control in turf, *Pseudomonas syringae* pv. *tagetis* on Canada thistle and other Asteraceae weeds and *Sclerotinia sclerotiorum* for control of Canada thistle, dandelion, and diffuse and spotted knapweeds [16-23]. While broad-spectrum activity is viewed as an advantage for a commercial product, whether it is biologically or chemically based, many chemical herbicides exhibiting this feature may be restricted as to application at particular growth stages of the crop without causing a certain level of crop injury [24]. Some of the host specificity traits of many biological herbicides may be advantageous since there is a greater assurance that nontarget and beneficial plant species will not be damaged and the bioherbicide can be applied at any growth stage of the crop without injury [14,24]. One of the benefits of DeVine® was that it persisted in the soil to provide long-term and residual activity; however, this same trait could also be considered a disadvantage if it creates problems for crop rotations where the crop may be a susceptible target to the bioherbicide pathogen. In addition, from an industry perspective, long-term residual effects can lead to reduced demand for repeat product sales, which can be exacerbated by the fact that the product has a small market potential in the first place [24]. These issues also hold true for chemical pesticides. In addition, DeVine® must be refrigerated and the product must be made-to-order 30 to 60 days prior to its intended use due to its low stability and thus has limited shelf life [24,25].

From a technological perspective, Collego® is capable of being economically mass produced in liquid culture fermentation at a commercial scale, but other fungal bioherbicides have not seen this same achievement since many of the prospective fungi cannot readily produce spores in liquid, but sporulate more readily in solid-substrate fermentation [18]. Unfortunately, high labour costs, inability to control cultural conditions and maintain sterile conditions have been associated with solid-state fermentation [26]. BioMal® was not commercialized in Canada due to technical difficulties to mass produce it cost-effectively [27]. Unreliable field performance, a reason for the lack of success of many post-emergent bioherbicides, has often been the result of the requirement for long periods of dew or leaf wetness by the microbial pathogen [12,14]. Although Collego® and DeVine® perform consistently and with high efficacy in the field, these early prototype models were used under relatively conducive conditions and thus required very simple formulations. Collego® was effective because the target weed inhabits rice paddies where high humidity is normally present [28]. In the case of DeVine®, it is a soilborne pathogen and subjected to less fluctuating temperature and humidity.

Weeds reviewed

The weeds reviewed in this paper were chosen from recent survey and work done in weed control of Madhya Pradesh region during my PhD work. I have selected the weeds in this review are very noxious weed of world, it is not only weed for India. The details are mentioned below:

Parthenium hysterophorus

A invasive and noxious weed *Parthenium hysterophorus* L. (*Asteraceae*) is a global presence and responsible for human and animal health problems like dermatitis, asthma and bronchitis. They have also causes agricultural losses and biodiversity. This noxious invasive species is considered to be one of the worst weeds as per Holm., *et al*. [29]. *Parthenium* weeds distribution is all over the world, in addition to its native range in North and South America and the West Indies [30]. The weed firstly pointed out in Poona (Maharashtra, India) by Professor Paranjape, 1951. It was firstly reported by Rao [31] as a new species in India. They have control by physical control which involves hand weeding but it is a time consuming and unpleasant job, causes health hazards to labours. Another physical approach is burning method. It is not a useful control strategy due to it requires large quantity of fuel and it destroys other economically crops growing near to it [32]. Another approach used for Parthenium control is chemical method but they have disadvantages causing environmental hazards and weed resistance for herbicides atrazine 2, 4-D, metribuzin, paraquat, trifluralin, diphenamid, and glyphosate [33,34]. Application of biocontrol method is ecofriendly and effective for controlling weeds through the use of natural enemies likes insect and microbes. The application of microbes as herbicide Rajak., *et al*. [35] taken a survey around Jabalpur (Madhya Pradesh) to collect diseased specimens of *Parthenium hysterophorus* and isolated suspected pathogens. He has collected more almost 25 fungal species and identified, in which *Myrothecium roridum* has shown most potential herbicide activity. Out of 25 genera of fungi, *Colletotrichum gloeasporides* f. sp. *parthenii* isolated from diseased seedlings of *Parthenium hysterophorus*, has shown very high mycoherbicidal potential [35]. The screening for bioherbicidal agent, two species of *Fusarium* viz. *F. oxysporum* and *F. solani* from he infected root/stem of *Parthenium hysterophorus*. these strains were evaluated, and they have caused severe wilting to fungi and shown good potential as biocontrol agents [36,37]. There are various parameters are tested to develop a suitable bioherbicide from indigenous fungi *Sclerotium rolfsii* like types of media, concentrations of inoculum and formulations to the seedlings of *Parthenium hysterophorus*. Maximum seedling mortality were shown in which mycelia propagules were used as inoculum [38]. The strain *Phoma herbarum* FGCC#75 Cell free culture filtrate were evaluated for its phytotoxic against *Parthenium hysterophorus* shoot cut, detached leaf and seedling bioassays. The result showed the presence of a toxic metabolite in the cell free culture filtrate, have responsible for phytotoxicity for Parthenium weeds. The phytotoxic metabolite has characterized and extracted with butanol, hexane, chloroform, acetone and ethyl acetate. The ethyl acetate based fraction having phytotoxic compound 3-nitro-1,2 benzene dicarboxylic acid (3-nitrophthelic acid) [39].

Lantana camara

Lantana camara is native to tropical and subtropical America, it is considered as serious and one of the world's top ten weeds [40]. From several years, survey of bioherbicidal agent for this weed were done in various country. The fungi collected during survey was *Aspergillus* spp., (*A. nidulans*, *A. niger*, *A. terreus A. fumigatus* and *A. flavus*) cell free culture of 21 days old fermented broth has shown significant herbicidal property against *Lantana camera* shoot cut bioassay. It was observed that cell free culture filtrate of different species of *Aspergillus* had varied degree of toxicity against *Lantana camera*. There was significant reduction in chlorophyll and protein content reported by Pandey., *et al*. [41]. Saxena and Paney [42] tested herbicidal substances secreted by microbes found that the culture filtrate of an indigenous isolate of *Alternaria alternata* SSLC # 103 exhibited marked phytotoxic effect against the weed *Lantana camera*. 41.62% and 52% change in biomass was recorded after 36 hours post-treatment and at 50% and 100% cell free filtrate concentrations respectively during the invitro whole plant bioassay. Partial purification of the cell free culture filtrate yielded four fractions, of which phytotoxicity resided in the Fraction A and it was a fatty acid. The shoot cut bioassay of this fraction caused more prominent phytotoxic damage when compared to cell free culture filtrate (CFCF). Singh [37] has tested two species of *Fusarium* viz. *F. oxysporum* and *F. monilifrome* which is isolated from infected leaf of Lantana. These two strains have shown very good results and causes wilting of weeds within 7 days application. It has taken as potential as bio-control agents for Lantana weeds.

Xanthium strumarium

Xanthium strumarium a annual *Asteraceae* family weeds invades roadsides, wastelands, riverbanks, farmland, overgrazed pasturelands. It is major weeds of maize, groundnuts, cotton and soya beans. It is toxic to animals. It is responsible for several agricultural, environmental and health problems in India [43]. It grows luxuriantly and seriously in infested paddy, sorghum and other kharif annual crop fields in Andhra Pradesh, Maharashtra, Rajasthan and Madhya Pradesh [44]. The weed is considered as one of the world's worst weed [40]. All the parts of the weed are highly toxic and allergic to humans and animals [45]. The major toxic substance in *Xanthium* is carboxyatractyloside which can kill hogs, cattle, goats, horses, sheep and poultry. Though the seed and seedlings contain the highest quantity of toxin, the whole plant can also be toxic [46]. The allelochemicals produced from different parts of the weed also inhibit the seed germination and seedling growth of many crops viz. Wheat, maize, pearl millet, chickpea, rapeseed, tobacco and lettuce [47]. Due to non-acceptability of conventional methods of control, the possibilities of its management through an indigenous strain of *Curvularia lunata* had been explored. A total number of 15 fungi were isolated from different parts of the weed *Xanthium strumarium*. The pathogens incited moderate to severe infection and caused significant damage to the weed. *Curvulavia lunata*, *Alternaria* spp., *Sclerotium rolfsii* and *Fusarium* spp., showed very high herbicidal potential [48]. Preliminary evaluation studies viz. Pathogenicity, herbicidal potential, safety to non- target organisms etc. carried out in laboratory conditions and the pathogen was found to have excellent mycoherbicidal potential against this weed. Similar results have also been reported by many other workers [49-51]. while evaluating the potential of *Alternaria crassa* for biological control of Jimson weed. Thus it can be boldly concluded that the mycoherbicidal agents can be applied in the field conditions for the biological control of weeds.

Cassia tora

It is an obnoxious, aggressive, annual and herbaceous that grows in most parts of India as a weed. It belongs to the Leguminosae family. It is an annual herb, 30–90 cm high which occurs as wasteland rainy season wild plant in India. It is generally distributed throughout India, Sri Lanka, West Indies, China and tropics. It was introduced originally from Tropical America [52] and is a very common weed all over the area along roadsides and in wastelands. It occurs in South-east Asia and the South-west Pacific where it is an important weed of pastures. It is troublesome weed of row crops in the southern United States and causes problems in India, Malaysia, Java, the Philippines and some pacific islands. It is a major weed of groundnuts, soyabeans, sugarcane, tobacco and pastures. Biological control of *Cassia tora* is being attempted in the United States. The available information on the natural enemies of these weed fungus *Alternana cassia* [53] is already being evaluated as a mycoherbicide in the USA. Two species of *Fusarium* viz. *F. oxysporum* and *F. monilifrome* were isolated from the infected leaf of *Cassia tora* and evaluated for biocontrol potential. The shoot cut bioassay of this fraction caused more prominent phytotoxic damage when compared to cell free culture filtrate (CFCF). Both the species caused severe wilting. The pathogens exhibited considerable potential as biocontrol agents [37].

Hyptis suaveolens

This weed belonging to *Lamiaceae* family is a native of tropical America and West Indies and was introduced in India as a Medicinal plant. It is a rigid herb of aggressive nature. This aromatic weed is now creating serious threats to biodiversity and resurgence of forest in Central India especially in Madhya Pradesh and Chattisgarh regions [54]. There are some reports where alcoholic constituents of the weed cause allelopathic effect on higher plants [55]. The spined burr catches in fur and clothing. Preliminary assessment of *Helminthosporium* sp. FGCC#74 as a potential mycoherbicide against *Hyptis suaveolens* was carried out by Pandey., *et al.* [56]. It was observed that the pathogen incited severe infection in the seedlings and the disease was initially characterized by the appearance of necrotic patches on seedlings and finally seedlings died. Singh [37] while screening the herbicidal substances secreted by microbes found that the culture filtrate of an indigenous isolate of FGCCW#43 exhibited marked phytotoxic effect against the weed *Hyptis suaveolens*. Significant herbicidal property of FGCCW#43 against Hyptis weed was seen in 21 days old fermented broth of cell free culture filtrate teste by shoot cut bioassay. It was observed that cell free culture filtrate of different species of *Fusarium* spp. have varied degree of toxicity against *Hyptis suaveolens*.

Sida actua

It is a common wireweed, a species of flowering plant in the mallow family, *Malvaceae*. It is native to Central America, but today has a pantropical distribution and is considered a weed in various regions. It can tolerate drought as well as high rainfall conditions. They are erect perennial shrubs up to 1.5 m in height, occurring on a wide range of soil types. They have yellow flowers and reproduce by seed. It is weeds in disturbed and cultivated areas [40]. Survey has conducted to isolate a potential fungal strain from *Sida acuta* and collection of various strains has done during research. Singh [37] reported *Fusarium* sp. FGCC#55 was showing phytotoxic damage on target weeds.

Antigonon leptopus

It is a perennial vine, lauded as an ornamental for its vigorous growth, and plentiful (usually) pink flowers, and even its ability to smother unsightly landscapes. When it is neglected, it can grow quickly over other vegetation, spreading beyond its area of introduction. Once established, it is difficult to eradicate because it produces many tuberous roots that can propagate vegetatively. Its fruits are buoyant, allowing for successful seed dispersal in water. Already it is classified as a Category II invasive [57-59]. For now, the best means of control is a combination of mechanical and chemical methods. Mechanical control is an effective means of controlling this plant but will not eradicate it [57]. The removal of aboveground tissue via cutting or mowing is not an effective method to eradicate plants because of the persistent, underground tuberous roots. To successfully control populations of this plant mechanically, the tubers need to be removed, and any reports repeatedly cut back [60]. Tubers can be found as deep as1 m in soil; therefore, deep tillage is necessary to remove tubers. Burning likewise can control plants above ground, and plants will produce shorter shoots after regrowth, but this is not a viable long-term option for control. Chemical control is a more effective long-term approach of managing its infestations. The fungal pathogen reported are *Colletotrichum, Pestalotia* [37]. Significant herbicidal property in cell free culture filtrate obtained from 21 days old fermented broth of *Fusarium* sp. FGCCW#43 against *Antigonon leptopus* was recorded by employing shoot cut bioassay technique [37]. It was observed that cell free culture filtrate of different species of *Fusarium* sp. had varied degree of toxicity against *Antigonon leptopus*.

Conclusion

Various microbial agent exists and preliminary research for metabolites characterizations has been conducted on various agent for two decades. Despite all this research and expense for development of microbial agent, there are very few have been successful and very few in the market. Lot of microbial agent did not success due to some reasons viz production problems, lack of stabilization of high titers following fermentation, lack of adequate shelf life of formulations under warehouse temperatures, lack of an economic viable delivery system, or loss of virulence of the product before reaching the target. To develop a better microbial herbicide, there is basic need to understand mode of action of bioagent or their products which is involved in host-pathogen interactions. They have leads to enhance the virulence of pathogen or suppress the host plant's defense. There are other factors like environmental conditions which is also play a basic role in the action of spores and products. The action of microbial metabolites (marasmins) could represent important in this condition. The availability of new methods for purification and quantification of product, structure elucidation, fermentation processing, synthetic production, formulation, knowledge of biosynthetic pathways and molecular tools for their transformation could give further support to the use of these natural metabolites as "helpers" of biological control strategies. The knowledge of toxin structure can permit the preparation of appropriate derivates and/or analogues that are essential to studies of structure-activity relationships, to the understanding of the mechanism of action, to the determination of the active sites of the toxins, and eventually to the production of related toxins having different biological properties. Many studies have shown that changing the active sites of microbial metabolites changes their biological activity. Much work remains to be done in the use of fungi toxins for weed control. With the development and correction of fewer techniques will provide good sources of microbial herbicide alternative for future generation weed control.

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